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SKIN PIGMENTATION IN RELATION TO ADRENAL CORTICAL FUNCTION*

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PIGMENTATION of the skin and mucous membranes constituted one of the cardinal features originally described by Thomas Addison in adrenal cortical disease (1). The pigment responsible has been shown histologically and chemically to be melanin (2), and many theories have been offered to explain the mechanism of the melanosis. An inability to utilize epinephrine precursors due to the loss of adrenal medullary func-

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tion, with resulting conversion of these materials to excess melanin, was an early theory (3). The adrenal was also suggested as a necessary link in the sympathetic control of neural crest melanoblasts, disease of the adrenal thus resulting in skin melanosis (4). Absence of adrenal steroidal hormones has been thought to cause pigmentation by interfering with storage of vitamin C, or to result in loss of reduced ascorbic acid secondary to the lowered sodium level (5). These theories have been discussed by Jeghers (2, 6), Sodeman (7) and Lerner and Fitzpatrick (8). Recently Calkins (9), Johnsson and Hogberg (10), Sulman (11) and Reifenstein (12) have offered the hypothesis that the pigmentation of adrenocortical insufficiency may be caused by excess intermedin or melanophore-expanding hormone, which may be produced in excess in association with adrenocorticotropic hormone (ACTH), or which may be identical with it. The occurrence of pigmentation as a complication of long continued ACTH therapy gives the problem an added clinical interest and lends force to the latter theory, although it is recognized that the chromatophores of human skin are not expansile.

Minor changes in skin color are difficult to assess by subjective observations, and photographic techniques also have great variability. The work of Rowntree and Brown (13) using the skin tintometer, followed by that of Dorno (14), Sheard *et al.* (15), Bode (16) and Williams (17) represents a series of increasingly successful attempts at objective analysis of skin color. The first detailed spectrophotometry of skin was made by Edwards and his associates (18) using the Hardy reflectance spectrophotometer under various conditions (19, 20, 21). Recently Edwards *et al.* (22) and Goldzieher *et al.* (23) have adapted spectrophotometry to the measurement of the ultraviolet reflectance of skin. Reflectance spectrophotometry is objective and quantitative and the curves obtained not only allow analysis of the component pigments, but provide permanent records for comparative purposes. The authors have studied changes in skin pigment reflectance in Addison's disease and in relation to ACTH and cortisone therapy in man.

MATERIALS AND METHODS

The Hardy machine was employed (24), in which monochromatic light is shone onto the skin and the percentage of light *reflected* at each wavelength —compared with that reflected from a standard magnesium oxide plate— is recorded automatically as the light is varied throughout the visible spectrum. Reflectance patterns of cheek, deltoid, nipple, palm and abdomen were obtained from 43 patients, including 10 patients with Addison's disease (6 of whom were studied before and after the administration of cortisone acetate), 9 patients before and after complete bilateral ad-

renalectomy, 8 patients who received daily intravenous or intramuscular doses of from 20 to 40 I.U. of ACTH and 4 patients receiving oral cortisone acetate in daily doses of from 300 to 500 mg.

Previous workers (18) in this field determined the *transmittance* spectra of various pigments of skin and assumed that these would be similar to the *reflectance* curves of the same materials in human skin. In order to provide a basis for interpreting changes in skin reflectance in terms of changes in individual pigments, an attempt was made to determine

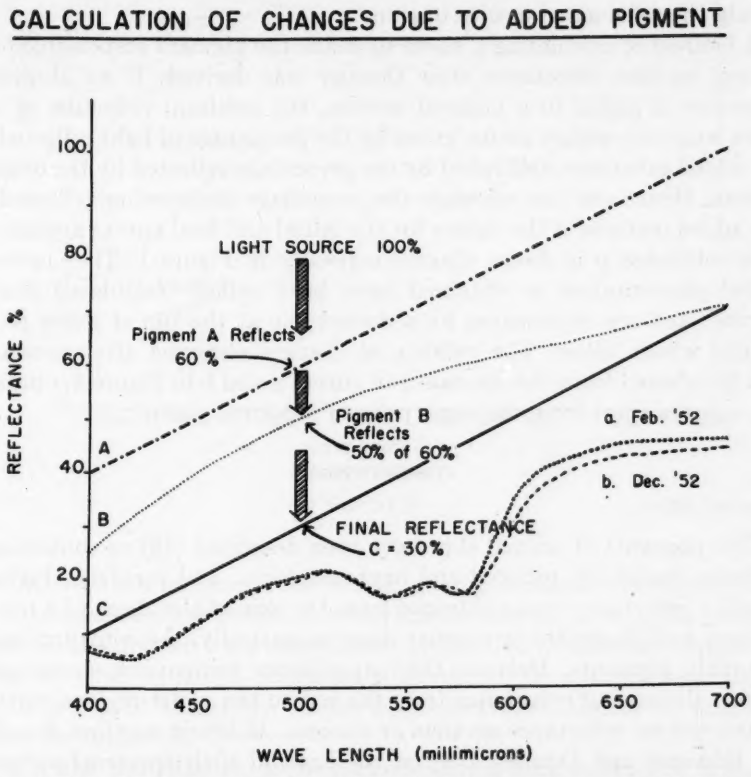

FIG. 1. Lines A and C are the initial and final reflectance curves produced by pigment A and pigments A plus B, respectively. The individual reflectance pattern of pigment B (line B) may be calculated as follows: At the wavelength selected for analysis, 500 mμ, pigment A reflects 60 per cent of incident light (initial curve A); and pigments A plus B reflect 30 per cent of incident light (final curve C). Since B equals C/A (*see* text), only 50 per cent of the light reflected by pigment A is reflected by pigment B (calculated curve B). Similar calculations at other wave lengths complete line B. It is apparent that the shape of the "calculated change curve" (line B) cannot be inferred from simple inspection of the initial and final reflectance curves (lines A and C).

directly their reflectance patterns. The reflectance spectrum of blood was investigated by dispersing it in a 40 per cent vegetable fat emulsion[1] which acted as a scattering agent. Melanin extracted from hair according to the method of Abel and Davis (25) was dissolved in normal sodium hydroxide and added to 25 per cent gelatin to form a clear filter, the reflectance spectrum of which was taken over a scattering background. The reflectance spectra of both blood and melanin differed from the transmission spectra, and probably because of the increased scattering which elevates the violet-blue-green end of the curves (18), resembled more closely the reflectance spectra of skin.

A method of calculating a curve to define the pigment responsible for a change in skin reflectance after therapy was derived. If an absorbing substance is added to a pigment system, the resultant reflection at any wave length is, within limits, given by the percentage of light reflected by the added substance multiplied by the percentage reflected by the original system. Hence one can calculate the percentage absorbed or reflected by the added material if the values for the initial and final curves are known. This relationship is shown diagrammatically in Figure 1. The curves of added pigmentation so obtained have been called "calculated change curves" and are represented by a dotted line at the top of many of the graphs which follow. The validity of changes observed after treatment can be inferred from the constancy of curves a and b in Figure 1, obtained ten months apart from the same palm of a normal control.

OBSERVATIONS

Normal skin

The pigments of normal skin have been described (18) as consisting of melanin, melanoid, reduced and oxyhemoglobin, and carotene. Figure 2 shows a reflectance curve obtained from the skin of the cheek of a normal subject and illustrates, somewhat diagrammatically, the contributions of the main pigments. *Melanin*, the major brown component, shows a uniformly decreasing reflectance from the red to the violet regions, without characteristic reflectance maxima or minima. *Melanoid* was first described by Edwards and Duntley (18) on the basis of their spectrophotometric findings. It has not been isolated, but was considered to be the substance responsible for the sharp downward depression of the reflectance curve in the near ultraviolet, which occurred in some areas with increased melanin content. These authors felt, from the association of this melanoid curve with melanin, and from their findings that melanoid tended to disappear

[1] Lipomul-Oral, obtained through the courtesy of Dr. R. P. Geyer of the Harvard School of Public Health and of The Upjohn Company, Kalamazoo, Michigan.

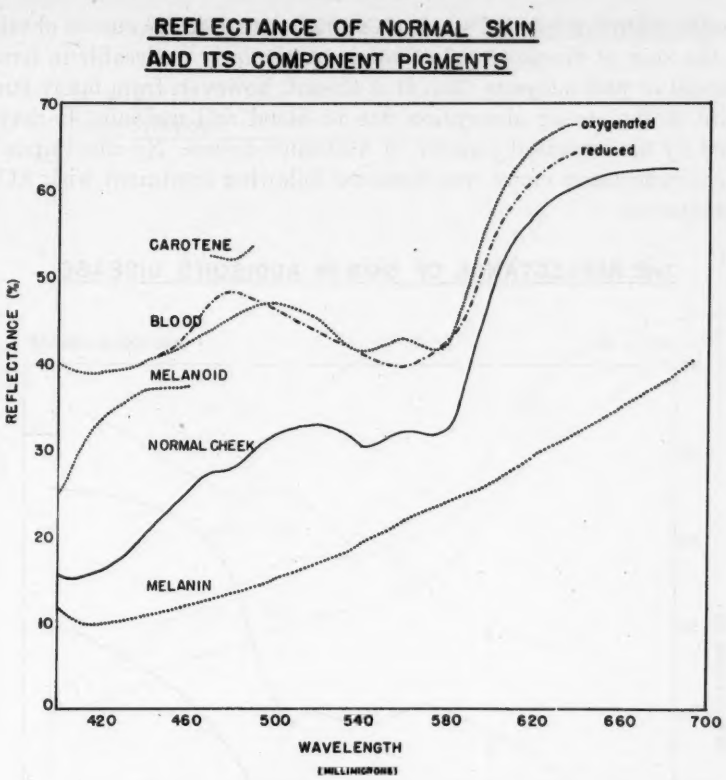

FIGURE 2

more slowly than melanin from skin tanned by exposure to sunlight (19), and that melanoid was probably a degradation product of melanin. Studies in our laboratory have shown that the melanin of human hair, following oxidation with hydrogen-peroxide, is converted to a compound which has an increased absorption in the near ultraviolet, similar to that described for melanoid.

The major contribution to the curve obtained from intact skin is made by *hemoglobin*. Oxygenated red blood cells suspended in fat emulsion show reflectance minima at 420, 545 and 575 mμ; with reduced hemoglobin the twin-dips at 545 and 575 are replaced by a single absorption band at 565. The pronounced upward slope of the hemoglobins in the near ultraviolet spectrum assists in differentiating between changes in blood concentration and decreased reflectance due to melanin or melanoid.

Skin *carotene* absorbs maximally in the region of 482 mμ. Absorption

due to this pigment is usually seen as a small depression in curves obtained from the skin of cheek or palm and is particularly noticeable in female, eunuchoid or pale subjects (20). It is absent, however, from many curves because of the strong absorption due to blood and melanin. It may be masked by the increased pigment of Addison's disease. No effect upon the carotene reflectance curve was observed following treatment with ACTH and cortisone.

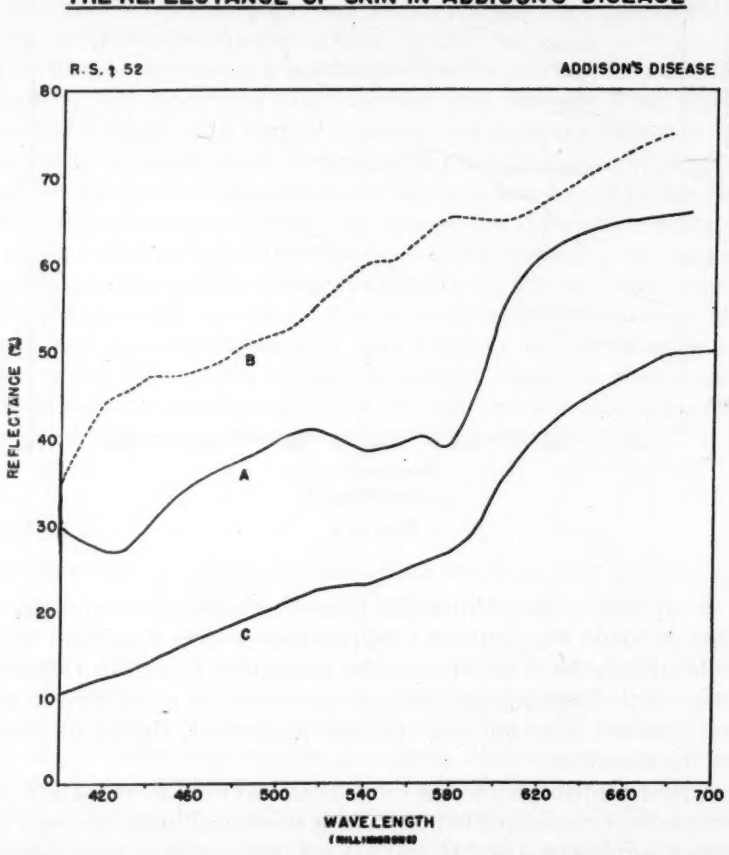

FIG. 3. Curve A—Vitiliginous skin. Curve C—Pigmented skin. Curve B—Calculated change-curve. The calculated change-curve B reveals an increase in melanoid (400–420 mμ) which is not readily apparent from curve A. The resemblance of the remainder of curve B to that of melanin is marked. The upward peaks in the region of the oxyhemoglobin maxima (545 and 575 mμ) indicate less absorption due to this pigment and therefore a diminished blood content of skin.

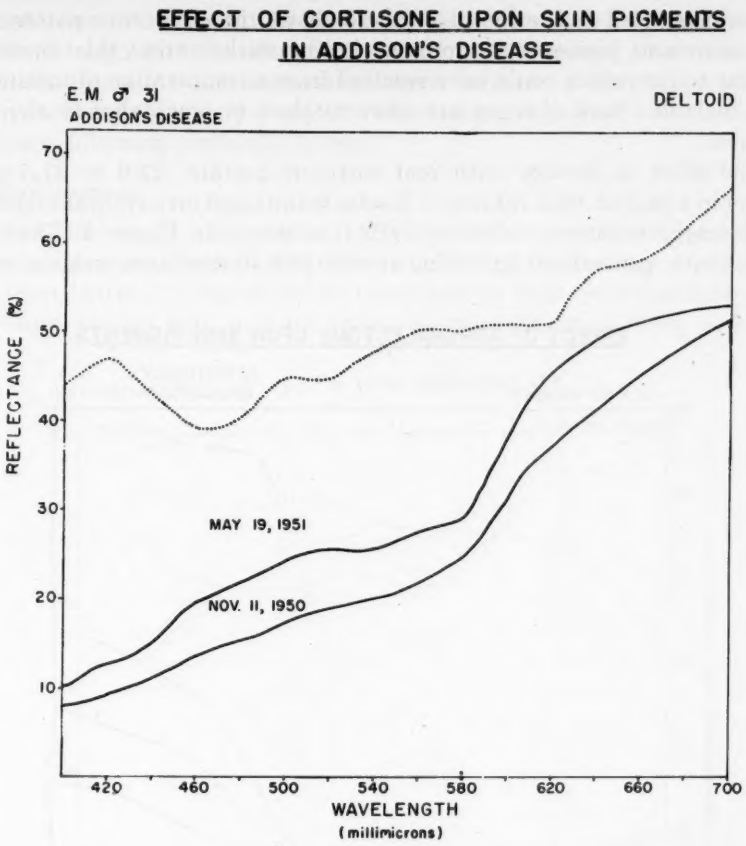

FIGURE 4

Addison's disease

The difference between the skin pigment in Addison's disease and in normal subjects can be seen in an exaggerated fashion by comparing adjacent pigmented and vitiliginous areas on the face of a patient with Addison's disease (Fig. 3). The distinguishing features of the curve in Addison's disease are: 1) the entire curve is lower, signifying a darker skin; 2) a fall, rather than a rise, is observed in the near ultraviolet, indicating the presence of melanoid pigment; and 3) the curve is flatter in the region 450 to 470 mμ and the twin-dips due to oxyhemoglobin are masked, a shift which may be accounted for by a predominance of venous blood with an absorption maximum at 565 mμ due to reduced hemoglobin, and by a smaller content of blood in the tissues. The calculated change-

curve illustrated at the top of Figure 3 shows the reflectance pattern of the *additional* pigmentation present in the darker area; this curve is similar to one which could have resulted from a combination of melanoid and melanin. Such changes are more marked in areas already rich in melanin.

The effect of therapy with oral cortisone acetate (25.0 to 37.5 mg. daily) in a patient with Addison's disease maintained on a constant dose of 11-desoxycorticosterone acetate (DCA), is shown in Figure 4. There is progressive, generalized lightening attributable to decreased melanin with

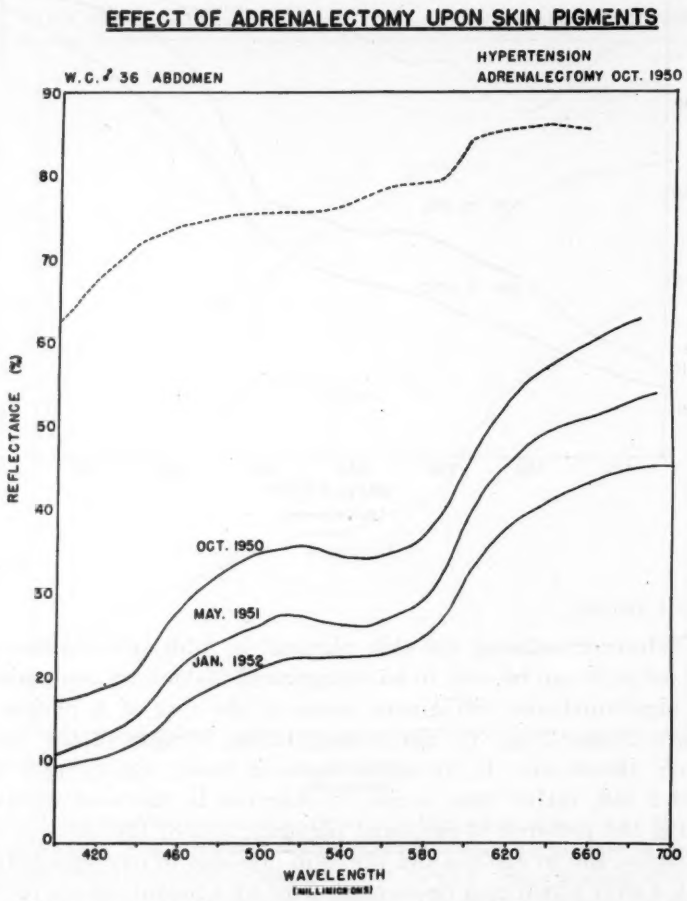

FIG. 5. The calculated change-curve shows an increase in melanoid, similar to that found in Addison's disease.

persistence of melanoid. The authors have observed a persistence of melanoid pigment in patients with Addison's disease, even after treatment with cortisone for eighteen months. Some increase in the oxyhemoglobin band was apparent in the majority of tracings of patients with Addison's disease, following cortisone therapy.

Adrenalectomy

The changes in skin pigment occurring after complete bilateral adrenalectomy are illustrated in Figure 5. These patients were maintained on from 25.0 to 37.5 mg. of oral cortisone acetate daily, plus supplementary sodium chloride and, in some cases, intermittent small doses of DCA.

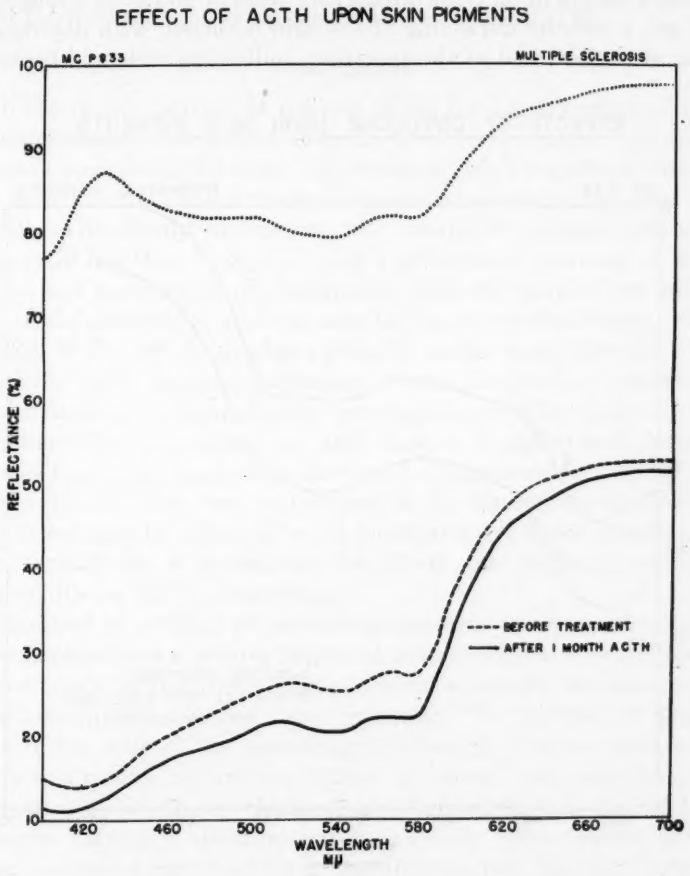

FIGURE 6

The degree of darkening observed in different patients varied considerably; the patient whose changes are shown darkened more than usual. The calculated change-curve has the same pattern of melanin-plus-melanoid seen in Addison's disease.

ACTH

Skin pigmentation is commonly seen as an accompaniment of long-term ACTH therapy (26, 27). This is in contrast to cortisone treatment in which increased pigmentation does not occur, except in cases of skin disease or locally around pellet implantation sites. Figure 6 illustrates the change in a 33-year-old woman with multiple sclerosis treated with ACTH. Following a month of daily intramuscular doses of 40 I.U. of a long-acting ACTH gel, a definite darkening of the skin occurred, with depression of the near ultraviolet end of the spectrum, indicating melanoid formation.

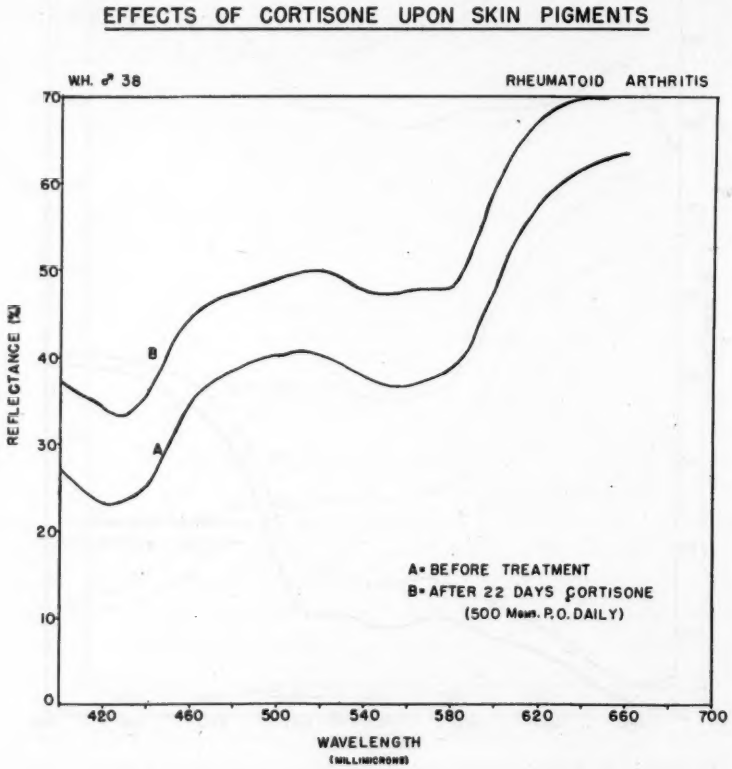

FIGURE 7

There also was increased melanin pigmentation, which was somewhat masked by a concomitant large increase in skin blood content. These changes were typical of all ACTH-treated patients and are emphasized by the calculated change-curves, which suggest a combination of increased content of blood, melanin and melanoid in the skin. Figure 7 shows, in contrast, the changes in a patient with rheumatoid arthritis during the oral administration of 500 mg. daily of cortisone acetate over a period of twenty-two days. In this instance lightening of the spectrum was observed with a decrease rather than increase in the downward slope from the red to violet, suggesting loss of melanin. There was little change in the region characterized by melanoid absorption. Two patients receiving cortisone for short periods showed some nonspecific darkening in one or two areas, but not the increase in melanin-melanoid seen after ACTH.

DISCUSSION

With the recent increase of interest in the hormonal control of human skin pigmentation, there is a definite need for accurate, reproducible methods of measuring skin color. The technique of skin spectrophotometry is well suited to the clinical study of conditions affecting the color of human skin. The Hardy instrument will accurately measure changes in reflectance of less than 1 per cent; this is well within the range of biologic variation and much more discriminating than the unaided eye. The accuracy of the machine is uniform over the entire spectral range, whereas the acuity of the eye diminishes markedly in the near ultraviolet where some of the most important changes occur. The greater sensitivity of spectrophotometric measurements may explain the discrepancy between our findings after adrenalectomy and those of Huggins and Bergenstal (28), who found no observable increase in pigmentation. Many of the changes reported here were not apparent on clinical examination, nor readily discernible by means of color transparencies under standard conditions. In addition to its extreme sensitivity, the method provides objective records for future comparison.

The method of analysis of reflectance patterns in terms of calculated change-curves allows a greater degree of differentiation between the contributions made by individual skin pigments, especially between melanin and melanoid, than has yet been reported. The method of studying changes in the ratio of the percentage of absorption at two points, used by Bode and others, is hardly adequate to identify the contributions of several pigments which are changing simultaneously (*e.g.*, during ACTH or cortisone therapy); identification is ordinarily quite feasible however with the calculated curves which are continuous over the entire spectrum (Fig. 8).

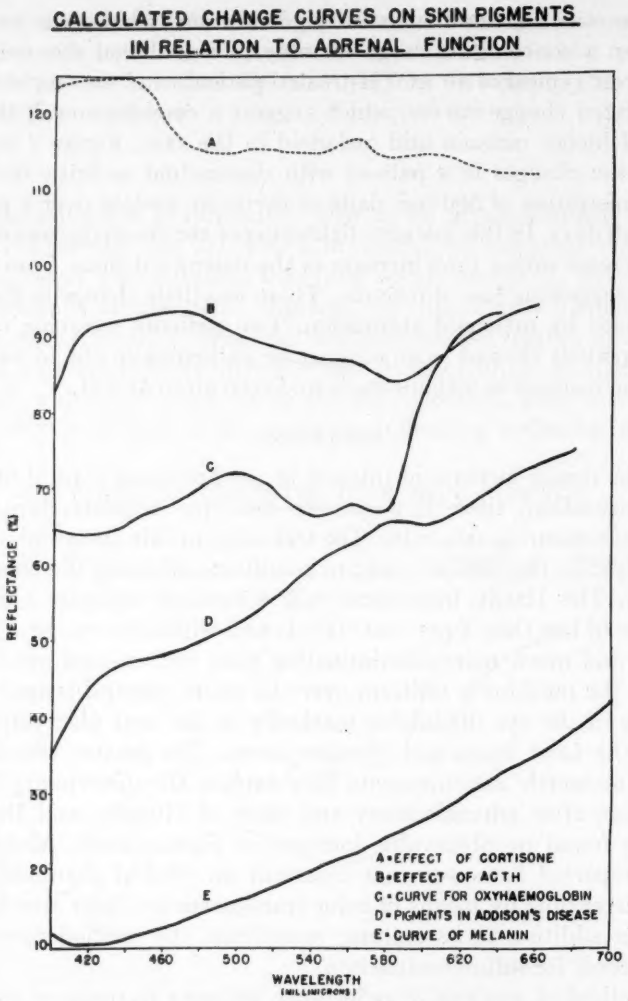

FIG. 8. Curve A shows a decrease in skin pigmentation after cortisone, the final curve having over 100 per cent of the reflectance shown in the pretreatment curve (Fig. 7); the major lightening occurs in the regions where melanoid and melanin absorb. The remaining curves have been spread out over the graph for easy visibility and are not related to the ordinate. In curve B, the great increase in blood flow almost masks the increase in melanin content.

In this study the authors have analyzed the reflectance changes in Addison's disease, and have been able to show their similarity to the pattern of increased pigmentation occurring after bilateral complete adrenallectomy or following prolonged administration of ACTH. ACTH-induced pigmentation was at first thought to be the result of adrenal cortical stimulation. Since a similar pigmentation is observed however in patients with Addison's disease in whom the pituitary is intact, whereas in hypopituitarism pallor is the rule, it seemed possible that the hyperpigmentation of both Addison's disease and ACTH administration might be a direct result of a pituitary, rather than an adrenal, hormone. Since the various lots of ACTH generally available have been demonstrated here and elsewhere to contain considerable quantities of intermedin, as determined by bioassay of hypophysectomized frogs, interest concerning the possible role of intermedin in the pigmentation associated with ACTH therapy has been heightened. Although intermedin has not been proved to be of physiologic importance in mammals, it is universally present in mammalian pituitaries, including that of man (29). The observation (30) that in amphibia intermedin promotes melanin synthesis (as well as chromatophore expansion) suggests the possible existence of a similar function in man. Excess pituitary secretion of intermedin brought about by adrenal insufficiency might thereby be responsible for pigmentation in Addison's disease. Similarly, cortisone could decrease pigmentation by effectively suppressing pituitary secretion; inasmuch as the lightening effect of cortisone is seen in patients satisfactorily maintained on DCA therapy, DCA might be considered ineffective as a suppressor of pituitary intermedin production. Whether ACTH and intermedin are identical hormones, as claimed by Johnsson and Hogberg (10) and by Sulman (11), remains to be proved; the studies of Astwood (31) and Li (32) indicate that separation is feasible. Studies on the effects of pure intermedin in man are awaited with considerable interest, now that reflectance spectrophotometry affords a sensitive and quantitative means of detecting the presence (or absence) of significant changes in the color of the skin.

SUMMARY

1. Studies of the skin color have been made with the Hardy spectrophotometer and the reflectance spectra of blood, melanin and melanoid have been analyzed *in vitro*.

2. A method has been derived for calculating a curve to define the pigment responsible for a change in skin reflectance after therapy.

3. The increased pigmentation observed in the skin of patients with Addison's disease appears to be due to a) increased content of melanin and melanoid; and b) decreased content of blood and oxyhemoglobin.

Cortisone reverses toward normal the pigmentary changes observed in Addison's disease.

4. Following bilateral complete adrenalectomy in man, despite maintenance cortisone therapy, an increase in pigmentation of the skin, comparable in every respect to that observed in Addison's disease, has been detected by this method of reflectance spectrophotometry.

5. Prolonged ACTH therapy in patients with intact adrenals causes changes in melanin and melanoid pigment similar to those encountered in Addison's disease, whereas the changes in blood content of the skin resemble those seen following cortisone therapy. Whether the increase in pigmentation following ACTH therapy is dependent upon the adrenal cortex or reflects the effect of contaminating pituitary hormones, cannot be stated at present.

CONCLUSION

The use of reflectance spectrophotometry offers an extremely sensitive and objective method of measuring and recording changes in skin pigmentation. Illustrations of its usefulness have been presented with respect to changes in the level of adrenal cortical function.

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THE EFFICACY OF A NEWER ESTROGEN, *TACE*, AS REVEALED BY VAGINAL SMEARS

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THE physiologic effect of most estrogenic substances administered orally is relatively brief. In the treatment and clinical management of certain syndromes of the climacteric, notably atrophic vaginitis-cystitis and osteoporosis, a long-acting estrogen would seem to offer definite advantages.

The possible value of such a compound in helping the menopausal patient to adjust to her normal postmature state has been described by Bickers (1). Large single crystals, microcrystalline suspensions, and pellets have been employed to extend estrogenic activity following a single parenteral administration. However, these techniques require either incision and insertion of a pellet or hypodermic injection of the active hormone. To obviate these difficulties, a long-acting oral estrogen has been sought and a new synthetic estrogen, TACE,[1] has been developed. The hormone is effective by mouth and because of its prolonged action, it has been termed an "oral implant."

TACE resembles diethylstilbestrol, hexestrol, and the natural estrogens in its estrogenic effect on the vagina, uterus and breast. It is unique and unlike other estrogenic substances, however, in that larger doses of TACE produce a prolonged effect because of the storage of the material in the body fat with a slow release of the estrogen (2).

Inasmuch as the subjective response of patients to treatment with estrogen is highly variable and not always trustworthy, the comparative efficacy of various estrogens on the basis of improvement in symptoms is difficult to appraise. Among the specific signs of the climacteric, the most noteworthy are: apparent vasomotor reactions, vaginal and bladder atrophy and demineralization of bone. The flushes are objective (although flashes, of course, are not). Not all women in the menopausal or postmenopausal era complain of vasomotor reactions, nor is osteoporosis a universal problem of hypoestrogenic women. Atrophic vaginal and vesical changes are the rule, however, after the menopause. Properly stained

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[1] TACE is the trade-mark of The Wm. S. Merrell Company, Cincinnati, for its brand of chlorotrianisene (tri-*p*-anisylchloroethylene).

vaginal smears will reveal the degree of atrophy secondary to estrogen deficiency. Vaginal smears are easy to obtain, stain and interpret and provide an accurate and more practical means for the clinical estimation of estrogenic effect than do endometrial or vaginal biopsies.

METHODS AND MATERIALS

Twenty-three postmenopausal, aged women who had received no estrogen for months or years previously were selected for estrogen study. They were divided into 4 groups and all received oral medication daily for three weeks. Each of the 11 patients in the first group received 6 mg. of TACE. A second group consisted of 5 patients, each of whom received 12 mg. of TACE. Two patients comprised the third group; these subjects each received 18 mg. of TACE. A fourth or control group of 5 patients was selected to receive a common synthetic estrogen, diethylstilbestrol, in a dosage of 0.5 mg.

Weekly vaginal smears were obtained from each patient during the period of therapy and for a number of weeks following cessation of medication. The spreads were stained by the standard Papanicolaou-Traut technique. The patients' clinical status, so far as improvement in vaginitis production of vaginal mucus and betterment of bladder function were concerned, was also noted. One comparison of dosage potency of the two estrogens was made by recording uterine withdrawal bleeding. A differential count of the epithelial cells of the vaginal smear was made in each case and an accurate statistically significant percentage interpretation of the cornified cells secured. This is possible if seven fields are viewed with a minimum of 400 cells counted, as suggested by de Allende and Orías (3). In making such a reckoning, a No. 8 ocular and a No. 6 objective are used. This combination provides a satisfactory field and easy differentiation is assured.

RESULTS

Inasmuch as all patients treated were elderly, ranging in age from 70 to 90 years, and their statements not always reliable, their subjective reactions to the drugs were not considered too seriously. However, many of the women described improvement in their sense of well-being and relief of vaginal and bladder discomfort with the medication. Only 1 patient reported nausea. She received 12 mg. of TACE daily. The gastric distress was not so severe as to cause discontinuation of therapy, however, and it was relieved by a change in time of administration of the estrogen from late evening to after meals.

Of the 11 subjects who each received 6.0 mg. of TACE orally per day for three weeks, uterine estrogen withdrawal bleeding occurred in 3. In

the group of 5 patients who received 12 mg. of TACE orally daily for three weeks, withdrawal bleeding occurred in 3. The 2 patients receiving 18 mg. of TACE orally each day for three weeks both experienced uterine bleeding after cessation of therapy. All 5 control patients who received 0.5 mg. of diethylstilbestrol daily by mouth for three weeks bled vaginally after termination of the hormone.

The oral depot effect described by Bickers (1, 4) required daily oral administration of 24 mg. of TACE for a period of at least thirty days in order to produce sufficient oral depot effect to permit 74 per cent of his

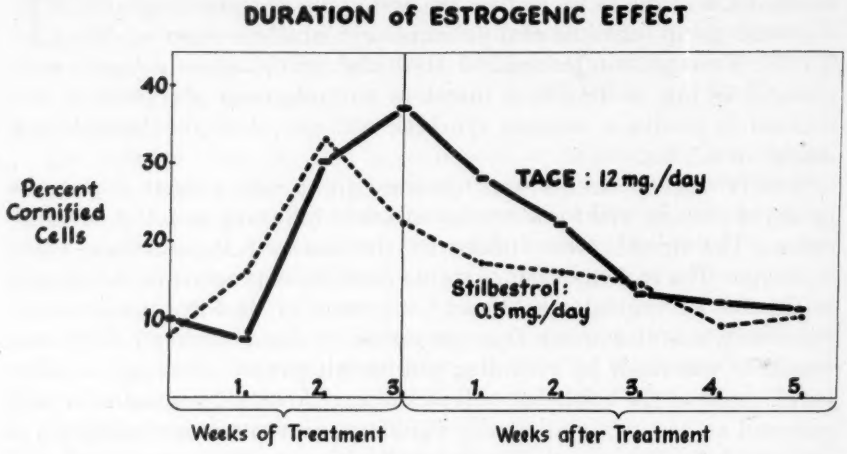

FIGURE 1

menopausal patients to obtain continuing relief from a single course of therapy (whereas only 38 per cent of the control group receiving natural conjugated estrogens required no subsequent endocrine therapy). In our experience, doses of less than 24 mg. of TACE daily are not adequate to produce a pronounced prolongation of estrogenic effect. Figure 1 shows that there is little, if any, increase in duration of estrogenic effect after withdrawal of daily doses of 12 mg. of TACE. Attention is called to the fact that doses even as low as 6 mg. of TACE daily produce the usual cornification of vaginal epithelium but do not produce the depot effect.

DISCUSSION

The synthetic estrogen, TACE, has been used in the arrest of carcinoma of the prostate (5–10) and in a variety of gynecologic syndromes (4, 11–13). It resembles diethylstilbestrol and hexestrol chemically in that the three compounds have two phenyl groups separated by two carbon atoms. These

carbon atoms are saturated in hexestrol and unsaturated in TACE and diethylstilbestrol. TACE also differs from the other two estrogens mentioned in that, instead of two hydroxy phenyl groups, it has two methoxy phenyl groups and two ethyls are replaced by a mexthoxy phenyl and a chlorine group (14).

Previous investigations have revealed that when patients have been difficult to maintain on diethylstilbestrol because of gastro-intestinal irritation, these same individuals could be easily maintained on equivalent doses of TACE without distressing nausea and vomiting (8). Laboratory animals receiving TACE were also studied concurrently. It was disclosed that the rats receiving TACE, unlike animals treated with diethylstilbestrol and hexestrol, did not present adrenal or pituitary enlargement, even when given large amounts of TACE over considerable periods of time (14–16).

The most intriguing observation hitherto recorded in animal experimentation involving TACE excretion is the prolonged effect of the estrogen following oral administration. It was demonstrated that this drug was stored in body fat, with a gradual release of the estrogen from lipoid depots over a period of more than ten days (2, 14). Hexestrol administration was not followed by storage and prolonged liberation of the estrogen. In this respect hexestrol is like other available estrogens.

CONCLUSIONS

TACE is a new synthetic estrogen which produces the usual estrogenic effects upon the senile vaginal mucosa when given orally to human subjects in a daily dosage of 6 mg. or more. In order to produce a pronounced prolongation of estrogenic effect after withdrawal, the dose must be something more than 18 mg. daily. The small doses employed in this study produce the usual subjective and highly gratifying effects associated with adequate doses of other estrogens.

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COMPARATIVE AND STATISTICAL STUDY OF VAGINAL AND URINARY SEDIMENT SMEARS

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I. INTRODUCTION

IN GYNECOLOGIC and endocrine practice, the examination of vaginal smears furnishes valuable information about the functional state of the ovaries, with regard to both trophic and cyclic activity.

In the year 1946, del Castillo, Argonz and Galli Mainini (1) proposed, for the same purpose, a new method capable of substituting in certain cases for the vaginal smear and based upon assays with the urinary sediment. These authors observed that the examination of urinary sediment cells, repeated in different stages of the sexual cycle, showed characteristic variations which, when the smears were made in the same manner, exhibited a "close parallelism" with the variations in the cells of the vaginal mucosa. When estrogen was administered to patients, either castrated or with the syndrome of rudimentary ovaries, identical modifications could be observed with the two methods.

With a view to observing those relationships more closely and in an attempt to determine statistically whether the "parallelism" mentioned by the original authors existed or not (permitting a comparison of the results of the two methods) the vaginal smear and the urinary sediment smear were carried out simultaneously upon 68 women.

The object of the present study, however, is that of obtaining uniform criteria as regards these investigations which have been so fruitful but which have been differently applied according to conditions, patients and the research workers' preferences.

II. MATERIAL AND METHODS

Data recorded during the observation of 68 women who were seen on the Service for Endocrine and Nutritional Diseases in the Italian Hospital "Garibaldi" and in the Clinic for Endocrine and Nutritional Diseases were used in this work.

Age and marital state: The present study includes patients whose ages varied from 5 to 54 years; most of the cases (60 per cent) were between

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21 and 35 years of age. Twenty unmarried and 48 married women were in the study.

Diagnosis: The 68 patients were divided into six groups, the details of which can be seen in Table 1A. In order to unify the material, a certain number of gynecologic disorders, endocrinopathies and nutritional diseases

TABLE 1. VALUES FOR MEAN PERCENTAGES OF SUPERFICIAL ACIDOPHIL CELLS IN VAGINAL SMEARS AND IN THE URINARY SEDIMENT SMEARS

A *Diagnosis*	No. of cases	Mean percentage ± St.e.	
		V.S.	U.S.S.
Sexual maturity	15	23.6± 4.38	15.2± 2.50
Oligomenorrhea	21	21.7± 3.76	16.3± 2.77
Functional amenorrhea	9	20.8± 8.70	14.9± 5.96
Organic amenorrhea	4	26.7±16.2	19.5±10.4
Climacterium	13	18.6± 5.15	13.3± 4.29
Induced hyperestrogenism	6	57.6± 2.85	40.1± 6.53
Total	*68*	*24.9*	*17.5*
Mean difference: 7.4±2.07(St.e.)		t:3.57	p <0.001

B *Analysis of covariance*

V.S. F:0.67 p>0.05 U.S.S. F:0.467 p>0.05

C *Regression formulae*

$\hat{x}$:6+1.08Y

$\hat{y}$:3.854+0.548X

Correlation r:0.77

D *Analysis of covariance of the regressions for each diagnosis compared to only one regression*

F:1.55

$F_{0.05}$:2.38 p>0.05

E *Analysis of variance of superficial acidophil cell level according to diagnosis*

V.S. F:3.82 F_{001}:3.34 p <0.01

U.S.S. F:3.56 F_{001}:3.34 p <0.01

V.S.: vaginal smear; U.S.S.: smear of the urinary sediment; St.e.: standard error; $\hat{x}$: estimated value of superficial acidophil cells in the vaginal smear; $\hat{y}$: estimated value of superficial acidophil cells in the urinary sediment; X: % of superficial acidophil cells in the vaginal smear; Y:% of superficial acidophil cells in the urinary sediment; F: variance ratio; p: probability of the result being due to random sampling.

on which we had performed the comparative study had to be eliminated; the number of patients in each category was too small to make the data significant.

Procedure: The patients arrived at the Hospital or the private Clinic in the morning. They brought with them a bottle containing the first urine of the day, which was immediately filtered. They were instructed not to practice any genital hygiene before the medical examination. A smear was made from the vaginal contents. Then the clinical and gynecologic examinations were carried out and the results recorded on a special card for this study.

Great care was taken during these examinations as regards the possibility of local affections, such as trichomoniasis or cervicitis.

Technique of vaginal smear: The material was always extracted by aspiration at the level of the upper third of the vagina, by means of a glass pipette, and placed on a clean microscope slide. In order to obtain irreproachable preparations we worked along the lines indicated in the technical works of de Allende and Orías (2) and of Di Paola (3). The material was immediately fixed in alcohol-ether (in equal parts) according to the Shorr (4) method; then it was mounted in xylol and balsam.

Technique of urinary sediment smear: We followed accurately the original technique (1–5), according to which the urine of the first micturition in the morning is filtered, and the material which remains on the filter paper is rubbed off and transferred to a slide. The material was fixed and stained by the Shorr (4) method and mounted in xylol and balsam.

Reading the preparations: We tried as far as possible to perform 400 readings in 7 different fields, since according to de Allende and Orías (2), in this way the error arising from a casual agglomeration in a field of only one type of cells can be avoided.

The results are expressed in percentage in accordance with the different types of cells. In order to simplify the study we have differentiated four fundamental classes and have eliminated, as noted by de Allende and Orías (2), all classifications which, even though more exact from the descriptive point of view, are of less practical value. The classification used in this work is the one suggested recently by Di Paola (3), based exclusively on staining affinity without referring to the cornification process, the nature of which is a matter of discussion. This author divided the cells observed in the vaginal smear into: superficial acidophils, superficial basophils, intermediate basophils and deep basophils.

In the sediment of the urine, too, these four fundamental cell types were detected, similar to those in the vaginal cells but not of the same structure.

As this work was carried out for clinical purposes, the author leaves the description and detailed differentiation of these cell types to the histologist.

It is sufficient to state that concerning their morphologic and staining conditions, it is perfectly possible to differentiate one from another.

In the urinary sediment as well as in the vaginal smear are described the presence of mucus, erythrocytes and leukocytes, the degree of cellular grouping, and the morphologic modifications which may occur. In order to present a more comprehensive scheme, no reference has been made to uterine, cervical and endometrial cells in the vaginal smear, or to other cells of the urinary tract in the sediment of the urine.

The extraction, staining and interpretation of the preparations have all been performed by the author.

III. RESULTS

We have selected the counts of superficial acidophil cells found by both procedures with the object of submitting them to statistical analysis, since they represent the index of fluctuations of estrogen activity. We have experimented with the originals (expressed in percentages) and wish to indicate that they have not been corrected by angular transformation. This arduous task did not seem justified, since many of the values were above 20 per cent, and the remainder very near this percentage; these, on their part, came from 400 cells counts, for which reason the margin of possible error was small. From the beginning it could be seen that there was almost constantly a clear correlation between the values for the superficial acidophil cells of the vaginal smear and those of the urinary sediment, determined out at the same time. The value of "r" (correlation) was highly significant, as can be seen in Table 1C.

The first step in the statistical analysis was to determine the average values with their corresponding standard errors, for each group into which the material was divided and for each one of the methods. Table 1A shows the results obtained, which confirm our first impression, namely, that the values of the means demonstrate clearly the correlation which exists between the two processes. The same Table 1 also illustrates the values which correspond to the general means.

It must be noted as a constant feature that the number of acidophil cells in the vaginal smear is always superior to that of the acidophil cells in the urinary sediment. The mean difference with its corresponding standard error can be seen in Table 1A.

The finding of such a constant mean difference and one of such statistical significance, suggested the possibility of establishing a regression between the values for superficial acidophil cells in the vaginal smear and those in the urinary sediment smear, which would enable us to convert those found by one method into those found by the other.

We began performing partial regressions of each one of the groups under

study but the analysis of covariance (Table 1D) showed that there was no significant difference between them; for that reason we elaborated only one, which included all the patients. Hence we obtained only one regression formula for each method. The respective formulae are illustrated in Table 1C.

The analysis of covariance (Table 1B) also showed that the means do not differ significantly from the regression line, *i.e.*, that there exists a constant relationship between the two methods, independently of the ratio of the cornified cells.

A persistent difference was observed between the number of superficial acidophil cells in the vaginal smear and in the urinary sediment smear, being always greater in the vaginal smear. This does not mean that one source is more sensitive than the other, as even with the said unequal levels, the differences which exist between the various diagnostic values have a similar significance with both sources (Table 1E) for the vaginal smear: $p < 0.01$, and for the urinary sediment: $p < 0.01$.

IV. COMMENT

As we have stated at the beginning, del Castillo and his collaborators concluded in 1946 that the examination of the smears of the urinary sediment, performed in women in different stages of the sexual cycle, showed characteristic changes which were in "close parallelism" to those of the vaginal smear. On that occasion, they also stated that estrogen substances produced identical modifications in the smears of the urinary sediment and of the vagina. They valued the method highly for its easy handling and, owing to the simple and practical technique, affirmed that it would take the place of the vaginal smear in many instances.

In 1949 the same authors demonstrated in a complementary work (6) that the cells of the urinary sediment originate from the lower part of the urethra (possessing similar characteristics to those of the trigonum vesicae) and that they are similar to, but not identical with, those of the vaginal epithelium; these too are affected by estrogen levels and vary in accordance with them. The authors mention the interesting considerations of Zuckerman (7–8), which support this conclusion. According to this investigator all the epithelial structures derived from the sinus urogenitalis (like the urethra), preserve the capacity of responding rapidly to female hormones by undergoing a process of stratification and desquamation.

These observations, therefore, have solved some of the problems of gynecologic and endocrine practice. It is well known that the obtaining of vaginal smears is often resisted by extremely shy women or children. Not only is this difficulty overcome but also one is enabled to obtain daily controls without too much inconvenience.

The setting up of regression formulae[1] makes it possible for the research worker to convert the values from one source into terms of values from the other source, thus permitting a comparison of assays carried out differently. The following example seems illustrative: supposing an investigator has obtained a value of 30 per cent for superficial acidophils cells obtained from the vaginal smear, and is desirous of finding out to which percentage this corresponds in the urinary sediment smear. Applying the pertinent formula, we have:

$$\hat{y}: 3.854 + 0.548X$$
$$\hat{y}: 3.854 + 0.548 \times 30$$
$$\hat{y}: 3.854 + 16.44$$
$$\hat{y}: 20.294$$

i.e., a value of 30 per cent for acidophil cells in a vaginal smear, corresponds after the applied formulae, to about 20.3 per cent in the urinary sediment.

Another process which enables the transformation of the values of one method into those of the other, is that of graphic conversion, as illustrated by the diagram in Figure 1. As may be seen, a 30 per cent value for superficial acidophil cells in the vaginal smear (x) corresponds to a 20 per cent value for the same type of cell in the urinary sediment smear. Therefore, the graphic process is rapid and sufficiently exact.

In addition, we have demonstrated that this relationship between the two methods is independent of the range of the values for superficial acidophil cells with which we work,[2] *i.e.*, of the estrogen level. In spite of the values of the means for the vaginal smears in these diagnostic studies being always superior to those for the superficial acidophil cells in the urinary sediment, there is no indication of any decrease in the sensitiveness of the one method with respect to the other; on the contrary, the analysis of variance proves that the sensitiveness is almost identical.

As a method of investigation of the estrogenic activity in women, we believe that the smear of the urinary sediment deserves the attention of the gynecologist and endocrinologist, because the information it furnishes is of equal value and sensitiveness to that of the classic vaginal smear.

From this we may deduce that the choice of the method will depend upon the particular circumstances of each case and not upon the unconditional preference for one of the methods, as both methods furnish similar information and appear to have the same sensitiveness. Moreover, it is

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[1] The regression formulae do not allow for values below 10 per cent. This is because the real regression line is probably curved (maybe sigmoid). If this error were avoided, there would occur an appreciable increase in the complexity of the regression formulae and, as a consequence, the method would be no longer practical.

[2] Within the limits of the means in this experiment.

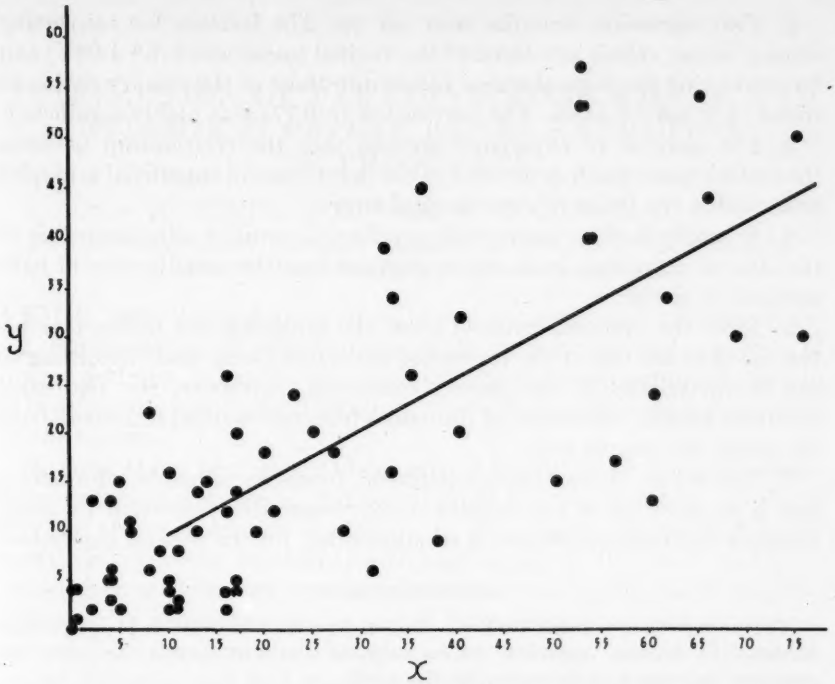

FIG. 1. *Dot diagram of the percentage of superficial acidophil cells*: x = vaginal smear; y = urinary sediment. If one does not wish to apply the regression formulae, this diagram enables the rapid conversion of the cornified cell values of one process into those of the other process, in a sufficiently accurate manner.

possible to transform the values of one into those of the other, in order to make them comparable, independently of the manner in which they are obtained.

V. SUMMARY AND CONCLUSIONS

Both vaginal and urinary sediment smears have been carried out on 68 women divided into six groups according to various diagnoses. A statistical study of the material has shown that:

1. In the various groups under study, there was a "parallelism" of the values for superficial acidophil cells obtained by each method. The average for acidophil cells in the vaginal smear was always higher than the average for the same type of cells in the smear of the urinary sediment. A significant mean difference was obtained: 7.4 per cent $\pm$ 2.07 (St.e.). The general mean was 24.9 per cent for the vaginal smear and 17.5 per cent for the urinary sediment.

2. Two regression formulae were set up. The formula for converting urinary smear values into those of the vaginal smear was $\hat{x}:6+1.08Y$; and for converting the vaginal smear values into those of the urinary sediment smear, $\hat{y}:3.854+0.548X$. The correlation ($r:0.77$) was highly significant.

3. The analysis of covariance showed that the relationship between the two methods was independent of the percentage of superficial acidophil cells, within the limits of experimental error.

4. An analysis of variance of the number of cornified cells, according to the clinical diagnoses, leads one to suppose that the sensitiveness of both methods is similar.

5. From the practical point of view, the foregoing fact makes possible the use of either one of the processes, the choice being made according to the characteristics of the patient concerned: moreover, the regression formulae permit conversion of the values from one method into those from the other, in a simple way.

6. The smear of the urinary sediment furnishes valuable information and is as sensitive as the vaginal smear; hence its application by gynecologists and endocrinologists is recommended, for the reasons mentioned.

Acknowledgment

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INTERACTION OF PARATHYROID HORMONE AND VITAMIN D ON THE RENAL EXCRETION OF PHOSPHATE*

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THE interrelationships of parathyroid hormone and vitamin D are important for the understanding of the pathogenesis and treatment of rickets and also to explain the apparently paradoxical action of vitamin D in hypoparathyroidism. This study attempts to explain their interaction on the kidney.

Vitamin D has been shown under certain conditions to increase tubular reabsorption of phosphate, and parathyroid hormone has been shown to decrease this reabsorption (1, 2). On the other hand patients with hypoparathyroidism may be treated with large doses of vitamin D with a resultant increased urinary excretion of phosphate and reduction of the concentration of phosphorus in the serum. More recently it has been demonstrated that parathyroid extract given intravenously increases the glomerular filtration rate and renal blood flow. (3) Crawford has recently suggested that elevation of serum phosphate stimulates parathyroid secretion (4).

METHODS

Inulin clearance was used as a measure of glomerular filtration in all but one of these experiments in which endogenous creatinine clearance was used. Inulin was measured by the method of Harrison (5), and creatinine by the Peters' modification of the Folin method (6). Phosphate was measured by the method of Fiske and SubbaRow (7).

Fluid was infused intravenously at as close to a constant rate as possible, by a gravity drip which flowed from a 300-cc. dispensing burrette; it was adjusted by means of a tunnel clamp. A priming dose of inulin was given, followed by a maintenance dose dissolved in 0.57 or 0.85 per cent saline solution during the equilibration period of thirty to sixty minutes and during the control periods. If a phosphate load was to be given, the maintenance inulin for those periods was dissolved in a solution containing 11.36 Gm. of disodium phosphate and 2.76 Gm. of monosodium phosphate per liter. In a few clearances there was an equilibration period after the start of the infusion of the phosphate solution. When parathyroid hormone was given, it was injected rapidly intra-

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venously through the same needle by means of a three-way stopcock attached to the haft of the needle. The parathyroid extract contained 100 USP units per cc. Vitamin D was given intramuscularly in doses of 600,000 units (1 cc.) or 1,200,000 units in oil, or as oily concentrates by mouth.

Blood was drawn for measurement of phosphate and inulin content at the midpoint of each period. The periods ranged from ten to thirty minutes in length. At the end of each period the bladder was washed out with both saline and air. An attempt was made to produce a water diuresis in the patients during the clearances. This was usually unsuccessful in the smaller children, and only one third of the clearances were carried out with urine flows of greater than 1 cc. per minute. All clearances were performed in the postabsorptive state. Sedation with barbiturates was usually employed.

The subjects were children between the ages of 2 weeks and 12 years on the wards of the Children's Hospital of Pittsburgh, with 1 exception noted subsequently. (Newborn infants may suffer a period of relative hypoparathyroidism (8, 9) and hence are not included in this group.)

The patients without demonstrable vitamin D deficiency were for the most part in the hospital for investigation of convulsive disorders, but others were convalescent from acute infections of the respiratory system. They had no evidences of renal dysfunction. In addition, studies were carried out on 2 patients with rickets due to vitamin D deficiency and on 1 patient with familial resistant rickets. One of the investigators served as a subject for the study on a normal adult.

RESULTS

The intravenous injection of parathyroid extract in doses of from 2 to 10 cc. produced an increase in phosphorus excreted per minute as expected. In addition, there was an increase in glomerular filtration. This finding confirms the work published by Handler *et al.* while this study was under way (3). The increase in glomerular filtration was often preceded by a short period of depression. This lowering of inulin clearance could be entirely masked by increasing the length of the initial period after parathyroid injection. Because of the increase in filtration the phosphorus re-

TABLE 1. THE EFFECT OF PARATHYROID EXTRACT ON GLOMERULAR FILTRATION AND TUBULAR REABSORPTION OF PHOSPHATE IN A NORMAL INFANT

Patient, age & wt.	Time (min.)	Inulin clearance (cc./min.)	Serum PO_4 (mg. %)	Phosphate			
				Filtered (mg./min.)	Excreted (mg./min.)	Reabsorbed (mg./min.)	Reabsorbed (mg./100 cc. GF)
Ge	10	23.4	5.5	1.29	0.26	1.03	4.4
6½ mos.	13	23.4	5.4	1.27	0.28	0.99	4.2
6.7 Kg.			*5 cc. parathyroid extract, i.v.*				
	10	21.1	—	—	0.23	—	—
	16	26.8	4.9	1.32	0.45	0.87	3.3
	14	31.5	4.7	1.49	0.68	0.81	2.6

TABLE 2. THE EFFECT OF PARATHYROID EXTRACT BEFORE AND AFTER THE ADMINISTRATION OF VITAMIN D IN NORMAL INFANTS AND CHILDREN

Patient, age & wt.	Time (min.)	Inulin clearance (cc./min.)	Serum PO_4 (mg. %)	Phosphate			
				Filtered (mg./min.)	Excreted (mg./min.)	Reabsorbed (mg./min.)	Reabsorbed (mg./100 cc. GF)
Gi	13	65.0	5.4	3.51	0.68	2.83	4.4
6 yrs.	10	64.0	5.4	3.47	0.69	2.78	4.3
17 Kg.			*5 cc. parathyroid extract, i.v.*				
	20	76.0	4.5	3.43	0.94	2.49	3.3
	16	80.0	4.3	3.45	0.99	2.46	3.1
			Repeated 23 hours after 1,200,000 units vitamin D, i.m.				
	22	60.0	5.2	3.12	0.43	2.69	4.5
			3.5 cc. parathyroid extract, i.v.				
	20	65.0	4.9	3.19	0.76	2.43	3.7
	15	69.0	4.6	3.18	1.03	2.15	3.1
Kr	21	11.0	6.5	0.72	0.17	0.55	5.0
2 mos.			*2 cc. parathyroid extract, i.v.*				
4.4 Kg.	18	13.8	6.4	0.88	0.20	0.68	4.9
	18	17.6	6.3	1.11	0.27	0.84	4.8
			Repeated 23 hours after 600,000 units vitamin D, i.m.				
	19	13.2	6.1	0.81	0.16	0.65	4.9
			2 cc. parathyroid extract, i.v.				
	15	17.9	5.7	1.02	0.35	0.67	3.8
	15	15.9	5.7	0.90	0.24	0.66	4.2
Sh	15	29.2	5.4	1.58	0.32	1.26	4.3
11 mos.	23	26.8	5.4	1.45	0.25	1.24	4.6
8 Kg.			*5 cc. parathyroid extract, i.v.*				
	19	28.3	5.4	1.52	0.30	1.22	4.3
	15	28.0	4.9	1.38	0.29	1.09	3.9
			Repeated 60 hours after 1,200,000 units vitamin D, i.m.				
	16	24.0	5.3	1.27	0.36	0.91	3.8
		26.2	5.6	1.47	0.31	1.16	4.4
	20		*5 cc. parathyroid extract, i.v.*				
	18	18.5	4.9	0.90	0.20	0.70	3.8
	15	44.3	4.5	1.95	.63	1.32	3.0

absorbed by the tubules per minute may not always diminish and in Handler's dogs it sometimes actually rose (3). Because of this change in the filtration rates, the rate of phosphorus reabsorption has been calculated in terms of 100 cc. of glomerular filtrate. This method of expressing the data also permits a comparison of tubular reabsorption in a 3-Kg. child with that in a 30-Kg. child or an adult. Table 1 gives the results of a typical study made on a 6½-month-old boy. The average increase in glomerular filtration rate in 6 cases was 31 per cent compared to 37 per cent found by Handler *et al.* in dogs (3).

When vitamin D was given intramuscularly in large doses before such a study, the effect of exogenous parathyroid hormone was as great or greater than previously. In illustration, Table 2 shows the results of clearance studies made with the administration of parathyroid hormone to 2

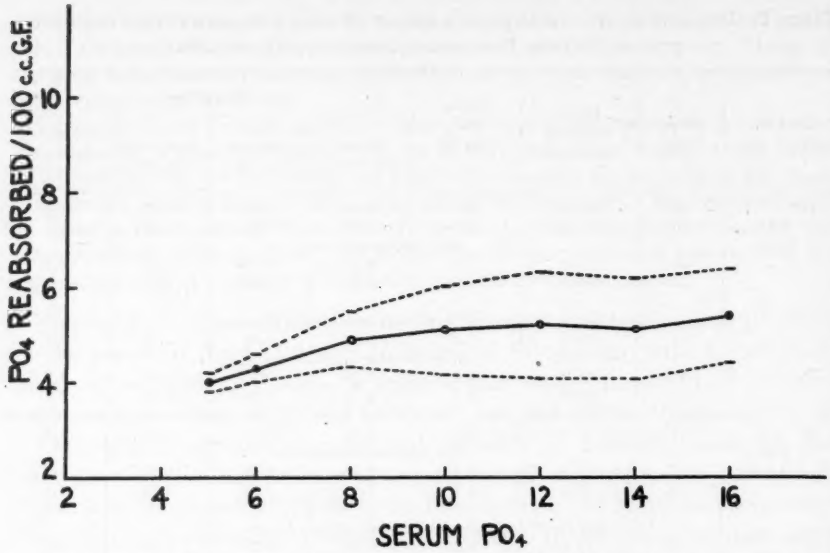

FIG. 1. *Tubular Reabsorption of Phosphate in Normal Children.*—Mean phosphate reabsorption per 100 cc. of glomerular filtrate is represented by solid line. Dotted lines represent one standard deviation from the mean. Serum phosphate is in mg. per cent.

patients both before and twenty-three hours after the injection of 600,000 and 1,200,000 units of vitamin D, respectively, and in 1 case both before and sixty hours after 1,200,000 units of vitamin D intramuscularly.

The results of 14 clearances in normal subjects, carried out with a rising level of serum phosphate produced by intravenous infusion of phosphate, are shown graphically in Figure 1. The reabsorption of phosphorus by the tubules rose slightly and then levelled off. This is similar to the results found in similar tests on dogs by Pitts and by Harrison (1, 2).

With the infusion of phosphate, glomerular filtration increased by an

TABLE 3. NORMAL PHOSPHATE CLEARANCE WITH A RISING LEVEL OF SERUM PHOSPHORUS IN A 10-YEAR-OLD BOY

Patient, age & wt.	Time (min.)	Inulin clearance (cc./min.)	Serum PO_4 (mg.%)	Phosphate			
				Filtered (mg./min.)	Excreted (mg./min.)	Reabsorbed (mg./min.)	Reabsorbed (mg./100 cc. GF)
Pa	15	57.2	5.4	3.08	0.92	2.08	3.6
10 yrs.				*Phosphate infusion started*			
28 Kg.	15	53.0	8.1	4.30	1.87	2.43	4.6
	14.5	77.5	10.1	7.82	4.62	3.20	4.1
	15	59.2	11.2	6.62	4.20	2.42	4.1
	15	58.5	13.0	7.60	5.39	2.21	3.8

TABLE 4. THE EFFECT OF VITAMIN D ON GLOMERULAR FILTRATION AND PHOSPHATE REABSORPTION IN 2 NORMAL CHILDREN, 2 CHILDREN WITH VITAMIN-DEFICIENCY RICKETS AND 1 WITH FAMILIAL RESISTANT RICKETS

Patient, age & wt.	Time (min.)	Inulin clearance (cc./min.)	Serum PO_4 (mg.%)	Phosphate			
				Filtered (mg./min.)	Excreted (mg./min.)	Reabsorbed (mg./min.)	Reabsorbed (mg./100 cc. GF)
Po (normal)	19	7.0	6.6	0.46	0.12	0.34	4.9
5 mos.	17	9.6	6.7	0.65	0.17	0.48	5.0
4 Kg.	15	11.5	12.5	1.44	0.66	0.78	6.8
	16	12.7	18.0	2.29	1.39	0.90	7.1
		Repeated 3 days after 600,000 units vitamin D, i.m.					
	10	8.2	5.6	0.46	0.13	0.33	4.0
	9	8.8	5.6	0.49	0.14	0.35	4.0
	19	9.3	6.4	0.60	0.11	0.49	5.2
	20	10.0	15.9	1.59	0.64	0.95	9.5
		5 cc. Parathyroid extract, i.v.					
	10	9.4	—	—	1.25	—	—
	15	10.6	20.7	2.20	1.57	0.63	5.9
St (normal)	12	29.9	5.6	1.68	0.42	1.26	4.2
16 mos.	10	30.2	5.6	1.70	0.42	1.28	4.2
11.5 Kg.	19	42.0	6.7	2.82	0.67	2.15	5.1
	14	37.6	10.0	3.76	1.91	1.85	4.8
	15	38.0	12.5	4.76	2.74	2.02	5.3
		Repeated 20 hours after 600,000 units vitamin D, i.m.					
	10	33.3	5.7	1.90	0.44	1.46	4.4
	17	33.2	6.1	2.03	0.54	1.49	4.5
	13	34.2	10.4	3.56	1.31	2.25	6.6
	15	34.7	13.9	4.82	2.54	2.28	6.6
Ger (resistant	10	58.5	3.3	1.94	0.37	1.57	2.7
rickets)	19	52.5	4.5	2.37	0.71	1.66	3.2
5 yrs.	13	58.2	6.7	3.91	2.08	1.83	3.1
20 Kg.	13	52.3	7.9	4.15	2.49	1.66	3.2
		Repeated 27 hours after 600,000 units vitamin D, i.m.					
	10	109.5	3.3	3.61	0.71	2.91	2.7
	10	101.0	3.3	3.34	0.71	2.63	2.6
	25	84.5	4.9	4.14	1 21	2.93	3.5
	12	75.0	7.4	5.56	2.79	2.77	3.7
	15	74.0	8.3	6.16	3.67	2.49	3.4
		Repeated 2 months later, after having taken 100,000 units vitamin D per day by mouth for this period					
	10	59.0	2.9	1.72	0.03	1.69	2.9
	10	52.0	2.9	1.52	0.07	1.45	2.8
	17	51.5	3.6	1.86	0.35	1.51	2.9
	20	50.5	7.2	3.54	0.16	3.38	6.7
	13	53.5	8.5	4.55	0.26	4.29	8.0
Co (vitamin-D-	21	34.7	3.3	1.15	0.19	0.96	2.8
deficiency	16	45.8	4.8	2.20	0.46	1.74	3.8
rickets)	12	30.0	10.2	3.07	1.58	1.49	5.0
13 mos.	11	34.5	11.9	4.10	2.51	1.59	4.6
10 Kg.							
		Repeated 23 hours after 600,000 units vitamin D, i.m.					
	19	29.6	3.4	1.01	0.15	0.86	2.9
	16	38.6	4.8	1.86	0.39	1.47	3.8
	16	38.2	8.8	3.37	1.51	1.86	4.9
	11	32.8	11.8	3.87	2.50	1.37	4.2
		Repeated 95 hours after original vitamin D. No further Rx.					
	10	50.0	3.6	1.80	0.19	1.61	3.2
	11	59.0	3.6	2.13	0.25	1.88	3.2
	18	51.0	4.8	2.45	0.39	2.06	4.0
	16	53.0	8.8	4.67	1.34	3.33	6.3
	18	63.0	14.0	8.80	2.14	6.66	10.6

TABLE 4—*Continued*

Patient, age & wt.	Time (min.)	Inulin clearance (cc./min.)	Serum PO_4 (mg.%)	Phosphate			
				Filtered (mg./min.)	Excreted (mg./min.)	Reabsorbed (mg./min.)	Reabsorbed (mg./100 cc. GF)
Repeated 18 days after original vitamin D. Had received 1,000 units vitamin D per day by mouth for two weeks							
	10	22.4	3.5	0.78	0.07	0.71	3.2
	16	28.2	4.0	1.13	0.19	0.94	3.3
	12	30.8	7.1	2.19	0.66	1.53	5.0
	11	35.2	10.4	3.66	1.53	2.13	6.0
Vo (vitamin-D-deficiency rickets) 10 mos. 7.2 Kg.	14	28.5	2.6	0.74	0.11	0.63	2.2
	14	24.0	2.6	0.62	0.09	0.53	2.2
	16	28.5	3.7	1.06	0.28	0.78	2.8
	13	25.3	7.1	1.79	1.09	0.70	2.8
	13	28.3	7.6	2.15	1.34	0.81	2.7
	Repeated 23 hours after 600,000 units vitamin D, i.m.						
	9	19.4	2.5	0.49	0.09	0.40	2.1
	9	19.0	2.5	0.48	0.09	0.39	2.1
	17	Lost	—	—	—	—	—
	13	26.5	11.1	2.94	1.68	1.26	4.8
	15	24.6	11.9	2.93	1.88	1.05	4.3
	Repeated 71 hours after original Rx. No further Rx.						
	14	27.8	3.7	1.03	0.22	0.81	2.9
	12	29.7	3.7	1.10	0.25	0.85	2.9
		Lost	—	—	—	—	—

average of 36 per cent in 14 patients. This rise was of brief duration and did not persist throughout the infusion. Table 3 gives the results of one such clearance performed on a 10-year-old boy.

The results of phosphate-loading experiments before and after the administration of vitamin D in 2 normal infants (Po and St), in 2 infants with rickets due to deficient vitamin D intake (Co and Vo) and in 1 boy with familial resistant rickets (Ger), are given in Table 4. Figure 2 records the reabsorption curves of the 2 normal infants.

The administration of exogeneous parathyroid hormone to one of the normal patients (Po) near the end of his second study was followed by increased phosphate excretion. There was no more increase in glomerular filtration rate. The effect of the exogenous hormone here is confused by the increasing phosphate load.

The patient with resistant rickets (Ger) had been admitted to the hospital for an osteotomy and had had no vitamin D intake for one month following his osteotomy and preceding his study. His rickets had formerly and has since been controlled with 100,000 to 150,000 units of vitamin D by mouth per day. A virtual doubling of the filtration rate took place twenty-three hours after the administration of 600,000 units of vitamin D to the boy with resistant rickets. An 80 per cent increase in glomerular filtration rate occurred four days after the same dose in 1 boy (Co) with vitamin-D-deficiency rickets. In the other children and in the latter boy,

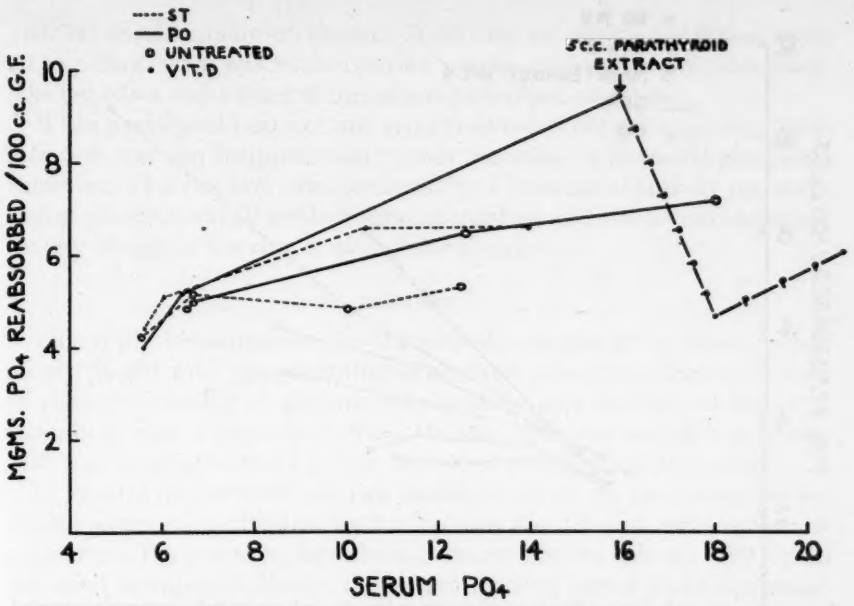

FIG. 2. *Tubular Reabsorption of Phosphate Before and After Vitamin D Administration in Normal Subjects.* The reabsorption of phosphate per 100 cc. of glomerular filtrate is plotted as before. The results in Patient Po are plotted with the solid lines, and in Patient St with the dotted lines. In both, the open circles represent reabsorption before vitamin D, the closed circles after administration of vitamin D.

twenty-three hours after the administration of vitamin D, the glomerular filtration dropped slightly or stayed the same in the control period or periods. This was also the case in those who did not receive a phosphate load (see Table 2). In the boy with resistant rickets the increase in glomerular filtration was not compensated for by an increased tubular reabsorption of phosphorus and as a result he excreted more phosphate. In contrast, in the case of the boy with vitamin-D-deficiency rickets, this increase in glomerular filtration rate was completely offset by the increased reabsorption of phosphate by the tubules and as a result he did not excrete more phosphate. The clearances were repeated 1) two months later in the boy with resistant rickets, while he was receiving 100,000 units of vitamin D per day by mouth, and 2) eighteen days later in the boy with vitamin-deficiency rickets, while he was receiving 1,000 units of vitamin D by mouth. At this time the situation had changed. The glomerular filtration rates were back to pretreatment levels, but the tubular reabsorption of phosphate had gone up in the boy with resistant rickets and stayed up

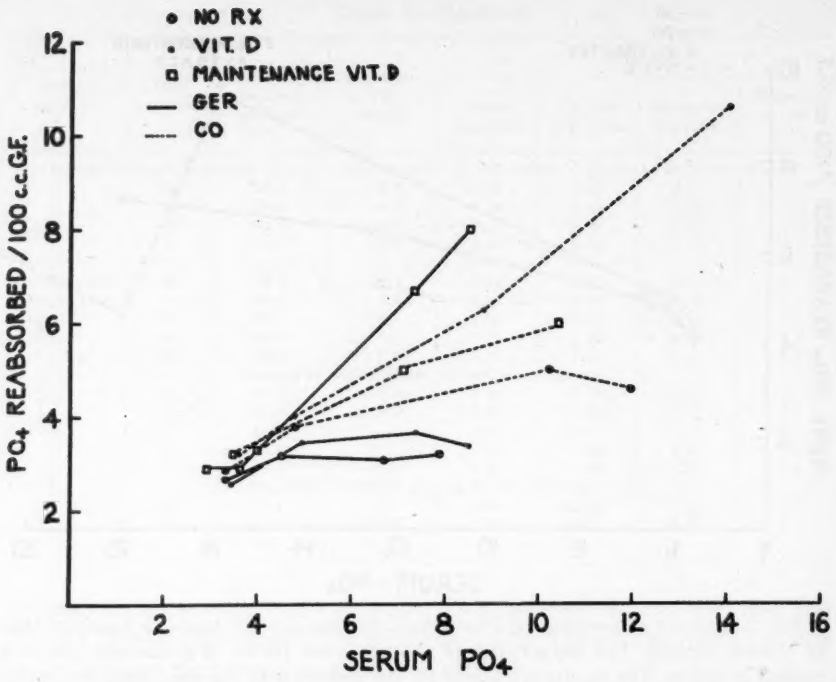

FIG. 3. *Tubular Reabsorption of Phosphate Before and After Vitamin D Administration in a Child with Vitamin-Deficiency Rickets and a Child with Familial Resistant Rickets.*— Reabsorption curves for Patient Ger with resistant rickets are plotted with solid lines; those for Patient Co, in dotted lines. Pretreatment curves in both are marked by the open circles; curves (one day for Ger and four days for Co) after intramuscular administration of 600,000 units of vitamin D are marked by closed circles; and the squares mark the results from the third clearances in both. These are two months later for Ger (while he was receiving 100,000 units of vitamin D a day by mouth) and eighteen days later for Co (while he was receiving 1,000 units of vitamin D per day by mouth).

in the other boy. X-ray examinations at this time showed healing of the rickets, though the serum phosphate level in both boys was still slightly low. When phosphate reabsorption was increased after administration of vitamin D in 2 patients (Ger and Co) maximal rates of reabsorption were not established, although previous loading tests had demonstrated apparent maxima for phosphate reabsorption. The reabsorption curves are plotted in Figure 3.

Similar studies on a 16-year-old boy with hypoparathyroidism are to be reported (10). These studies also show an increase in glomerular filtration rate of approximately 64 per cent following the administration of

100,000 units or more of vitamin D per day by mouth for fifteen days. At this time there was no increase in tubular reabsorption of phosphate. The net effect was a marked increase in phosphate excretion.

When parathyroid extract was given to this last patient in doses of from 2 to 5 cc. per day intramuscularly there was also an increased phosphate excretion. This however, was produced by a lowering of tubular reabsorption of phosphate at all levels of serum phosphate and was not accompanied by any change in the rate of glomerular filtration.

DISCUSSION

This study is complicated by the necessity of giving the vitamin D as an oily liquid with the possibility of variable absorption. Since the dose of vitamin D acting at any one time in large part decides the action of vitamin D, this is important. None the less, there are certain hypotheses that can be substantiated by the facts that emerge from this study.

1. Parathyroid extract has two modes of action on the kidney. It increases glomerular filtration and it reduces the tubular reabsorption of phosphate. These actions have been demonstrated in animals (1, 2, 3). In the usual therapeutic dosage, the second mode of action is the significant one.

2. Vitamin D apparently also increases glomerular filtration under certain circumstances. In addition, it increases the tubular reabsorption of phosphate. At present we believe it does the latter by inhibiting parathyroid secretion. It is possible that a more correct statement is, that it makes a higher level of serum phosphorus necessary in order to stimulate parathyroid secretion. It has been shown previously that tubular reabsorption of phosphate increases when rachitic dogs are given vitamin D (2). This increase in tubular reabsorption accompanies a rise in the level of serum phosphate. In this study phosphate reabsorption increased after vitamin D administration also, and a maximal rate of reabsorption could not be demonstrated in 2 cases (Ger and Co), whereas it had been demonstrated before vitamin D administration. In addition, the secretion of parathyroid hormone apparently establishes the maximal rate of tubular reabsorption of phosphate when a phosphate load is given (10).

Since vitamin D did not prevent the usual action of parathyroid extract when the extract was given after the administration of vitamin D, it does not appear to affect phosphate reabsorption by blocking the action of parathyroid hormone in any way. Since vitamin D in the absence of adequate parathyroid hormone in the patient with hypoparathyroidism did not increase tubular reabsorption of phosphate, we do not feel that it affects reabsorption by acting on another locus in the kidney, increasing

reabsorption more than parathyroid hormone lowers it. There is still the objection that the doses of vitamin D here used were too small or those of parathyroid extract too large. The doses of vitamin D were at least large enough to exert its effect in opposition to endogenous parathyroid hormone. Amounts of parathyroid extract as small as 2 cc. were administered and the doses used produced an average change in glomerular filtration rate which was less than that produced presumably by endogenous parathyroid hormone secreted in response to the stimulus of a rising level of serum phosphate. It is, therefore, believed that vitamin D directly or indirectly inhibits the secretion of parathyroid hormone.

It is possible now to explain speculatively the action of vitamin D in hypoparathyroidism, rickets and perhaps in resistant rickets.

In hypoparathyroidism, parathyroid hormone can increase phosphate excretion by its two actions on the kidney. It can both decrease phosphate reabsorption by the tubules and increase phosphate filtration, although we do not believe the latter mode is clinically significant. Vitamin D increases phosphate excretion in hypoparathyroidism, since in the massive doses used it increases the filtration of phosphate. Since in this disease there is no parathyroid effect to negate, the vitamin D has no effect on phosphate reabsorption. (There is an alternative explanation which is valid for this one situation. Tubular reabsorption of phosphate may be considered to be maximal in the absence of parathyroid hormone. Hence, vitamin D cannot increase phosphate reabsorption.) The action of large doses of vitamin D given over a long time on glomerular filtration has not been studied.

In cases of rickets, vitamin D inhibits parathyroid hormone secretion. Phosphate reabsorption is thus permitted to increase. The doses of vitamin D usually used to treat rickets do not elevate the glomerular filtration rate.

Vitamin D and parathyroid interaction may be the same in resistant rickets as in vitamin-deficiency rickets. Nevertheless, the alternative possibility must be considered that the amount of vitamin D required to inhibit parathyroid effect, relative to that required to raise glomerular filtration rate, is greater than in normal individuals or in individuals with vitamin-deficiency rickets.

These findings also permit the speculation that small doses of vitamin D may produce tetany in the rachitic child by causing increased phosphate reabsorption before calcium absorption from the alimentary tract is increased. On the other hand, massive doses of vitamin D may not cause tetany because the increased tubular reabsorption of phosphate is offset by increased filtration.

The absolute amounts of phosphate reabsorbed as reported herein, may be inaccurate for two reasons. The rapidity of the rise in the level of serum phosphate may be associated with significant amounts of nonfilterable phosphate. Secondly, there may be a lag in response to the rapidly rising loads and an error in assuming the average level of serum phosphate. For instance, we believe the slight rise in the rate of tubular reabsorption of phosphate before it levels off may be an artefact. There was, however, no noticeable difference in those cases in which the serum phosphate level was elevated more slowly. In any event the possible errors should not affect the validity of the changes in reabsorption with which this study is concerned.

SUMMARY

Parathyroid extract when given to humans has been shown to increase the glomerular filtration rate as well as to decrease tubular reabsorption of phosphate. The former may represent a true hormonal effect and not that of a contaminant, as this effect is seen when an intravenous infusion of phosphate raises the level of serum phosphate.

Vitamin D has been shown under certain circumstances to increase the glomerular filtration rate. Vitamin D has been shown not to inhibit the effect of exogenous parathyroid extract.

It has been suggested that vitamin D inhibits the secretion of parathyroid hormone.

Hypotheses have been suggested to explain the apparently paradoxical actions of vitamin D in rickets and in hypoparathyroidism.

Acknowledgments

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RARE DWARFISM WITH CHRONIC HYPOGLYCEMIA AND CONVULSIONS*

OBSERVATIONS WITH 1) ACTH AND CORTISONE, 2) ACTH AND THYROID

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INTRODUCTION

THE association of chronic hypoglycemia and convulsions in children with extreme dwarfism and retarded skeletal maturation is most unusual (1). This communication is concerned with 2 dwarf siblings, in whom hypoglycemic episodes developed shortly after birth; and an investigation of the type of dwarfism, the pathogenesis of hypoglycemia, and therapeutic methods for stimulation of growth and alleviation of chronic hypoglycemia. Observations on the use of ACTH with cortisone and ACTH with thyroid are now reported.

CASE REPORTS

Case 1

L. L., a 12-month-old white male was first seen at the Children's Memorial Hospital on September 21, 1942, because of failure to gain weight and recurrent convulsions. He was born on September 9, 1941, and weighed 6 pounds 10 ounces and was 19 inches long at birth. At the age of 2 days his skin had a greenish yellow color. The mother's blood was type A, Rh-positive and the child's blood was type O, Rh-positive. He appeared very sluggish and unresponsive, and the extremities were always cold and moist. At the age of 5 months he had his first convulsion. This occurred suddenly in the early hours of the morning and was associated with cyanosis of the lips and nose. Subsequently such episodes occurred about once a month. In addition, there were frequent "spells" characterized by listlessness, sweating and limpness without loss of consciousness. These would generally follow periods of poor feeding or vomiting and upper respiratory infections with anorexia.

The father, aged 37, and the mother, aged 25, were in good health and of average height and development. A complete family history was not obtainable. There had been a full-term infant, born on December 2, 1942, who died after 27 hours with deep cyanosis; an autopsy was not performed.

On examination, the child weighed 12 pounds 6 ounces and was 24 inches long. The temperature vas 97.2° F. The ratio of upper to lower measurements (vertex to symphysis

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and symphysis to heel) was 1.7 to 1. The circumference of the head was 17½ inches and of the chest, 15 inches. There was a pale icteric tint to the skin, which felt cool, moist and velvety. The facies had a cretinoid appearance with scant hair on the scalp and eyebrows. Both fontanels were wide open; the anterior measured 4 in. × 4 in. and the posterior, 1 in. × 1 in. The tongue was thick and protruded from the mouth. One lower incisor was present. The heart tones were heard poorly and there was a soft blowing murmur over the precordium. The edge of the liver was palpable just below the costal

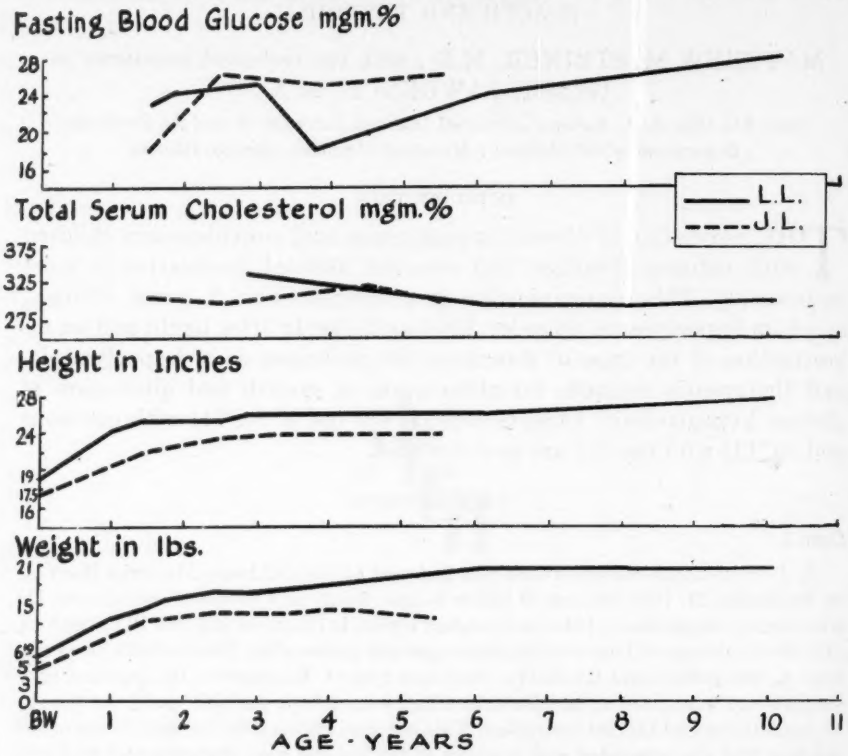

FIG. 1. Course of weight, height and chemical determinations in 2 dwarf siblings, L. L. and J. L. Note continued marked retardation in weight and height, elevated serum cholesterol level, and fasting blood glucose levels never above 28 mg. per cent.

margin. There was a minute penis and no testes were palpated in a small wrinkled scrotum. The nails were poorly developed and the edges were rolled. The musculature was flabby and had a doughy consistency. The superficial and deep reflexes were sluggish but there were no abnormal neurologic findings. He was unable to hold up his head and he did not sit with support, or stand.

Subsequently he was followed in the Out-Patient department and in the hospital. The course of the weight, height and chemical determinations is illustrated in Figure 1. His first hospital admission was at the age of 18 months on March 24, 1943. He weighed

14 pounds and was 25 inches long. There were no significant changes from his condition at 12 months. He had 2 lower incisors. A moderate microcytic anemia was present. The icterus index was 7 units and the concentration of total serum cholesterol was 320 mg. per cent. Roentgen examination showed one small carpal center in the wrist. There was slight osteoporosis of the long bones, and neither epiphyseal femoral head was present. The fasting blood glucose level was 22 mg. per cent. Thyroid substance ($\frac{1}{16}$ grain) was given daily for four days but hypoglycemic symptoms developed, with rapid loss of weight. At the age of 29 months, thyroid therapy was again attempted and he was given 1/20 grain daily for ten days. At the end of that time he had lost 2½ pounds and the mother reported frequent spells of limpness, cyanosis, sweating and almost daily twitchings. At the age of 3 years and 9 months he was hospitalized in coma with deep cyanosis and Cheyne-Stokes respirations. The blood glucose level was 18 mg. per cent. He responded favorably to artificial respiration and intravenous glucose solution. He had 2 upper and 2 lower incisors. The level of total serum cholesterol was 294 mg. per cent, and of total serum carotenoids, 1,000 I.U. per cent. At the age of 8 years there was no uptake of administered radioactive iodine by the thyroid, and there was no change in skeletal maturation.

At the age of 10 years and 2 months he was hospitalized for further study to determine the effect of ACTH on the concentration of blood glucose and the tolerance to thyroid under this influence. In the intervening years he had had repeated short convulsive seizures associated with fever, anorexia, respiratory infection and vomiting or diarrhea. It is notable that he had gained only 3 pounds and had grown only 2 inches in approximately 7 years! The dentition was similarly markedly retarded, so that at this time he had only the deciduous teeth. Despite the yellow tint of the skin the icterus index varied from 12 to 13 units; the color was probably the result of the high carotenoids in the serum. The values for total serum cholesterol ranged from 294 to 393 mg. per cent. The fasting blood glucose level was below 30 mg. per cent. He was now able to pull himself to a sitting and standing position and could walk around the crib. The temperature varied from 96.4 to 97.8° F.

On admission (Fig. 2) he weighed 20 pounds and was 27½ inches long. The measurement from vertex to symphysis was 17 inches and from symphysis to heel 10½ inches; the span was 26 inches; the circumference of the head was 18 inches, of the abdomen 19 inches, and of the chest 16½ inches. The penis measured ½ in. × ½ in. and no testes were palpable. The anterior fontanel was 1 in. × 1 in. and the posterior fontanel was closed. The hair of the scalp and eyebrows was sparse and thin. The tongue protruded and there was constant drooling. Dental survey by Dr. Gordon H. Rovelstad revealed eruption of all primary teeth. "The teeth were pearly in color and unusually small. There was no occlusion and in some places the gum tissue overlay the occlusal surfaces. There was no apparent function or actual occlusion of the teeth. The eruption age was that of a 2-year old. Roengten exam showed all primary teeth fully calcified (crown and root) both enamel and dentin. Also, the tooth buds of 1st molar, incisors, bicuspids and 2nd molar of the permanent teeth are clearly visible. The calcification age was approximately that of a 5 year old and would represent a truer dental age. There also appeared to be a lack of alveolar bone development in both maxilla and mandible." The neck was short and thick and there was a prominent fat pad over the shoulders. The thyroid gland was not palpable. The nipples were minute. There was no umbilical hernia. The liver and spleen were not palpable. The nails appeared atrophic and did not reach the end of the digits. The patient was able to sit without support and could pull himself to the standing position with some aid. He was able to take a few steps by holding on to the side of the crib

or the hand of the examiner. He would make clucking sounds and appeared to respond to sounds by grimacing or smiling. Roentgen studies of the long bones revealed two small carpal centers in the wrist and a small epiphyseal center in the femoral head (bone age about 6 months).

FIG. 2. *L. L.*, aged 10 years, weight 20 pounds, length 27½ inches. Note sparse hair of scalp and eyebrows, cretinoid facies, protruding tongue and very small genitalia. Only deciduous teeth present. Bone age 6 months.

J. L., aged 5½ years, weight 13½ pounds, length 24 inches. Note sparse hair of scalp and eyebrows and cretinoid facies. Only 12 deciduous teeth present. Bone age 3 to 4 months.

Case 2.

J. L., an 18-month-old white female (the younger sister of L. L., Case 1) was admitted to the Children's Memorial Hospital on October 28, 1947, because of convulsions, anorexia, failure to gain weight, marked pallor and persistent constipation. She was born

on April 3, 1946, the third child of normal parents, and weighed 5 pounds and was 18 inches long. She appeared jaundiced on the second day; the blood was type O, Rh-positive. Because of cyanosis and dyspnea on the third day she was placed under oxygen. She fed poorly and convulsive seizures developed, starting from shortly after birth to the age of 3 months, averaging 5 to 6 seizures daily. At the age of 2 months, desiccated thyroid (dose?) was given but she showed more frequent and severe convulsions. From the age of 3 months, there had been no severe convulsions, but instead there were frequent "spells" characterized by limpness, staring and upturning of the eyes, and transient cyanosis. These spells occurred particularly during periods of anorexia or during upper respiratory infections.

On examination she weighed 12 pounds and was 22 inches long. The ratio of upper to lower measurement was 1.7 to 1. The skin was cold, smooth and cyanotic in addition to having a peculiar yellow tint. The circumference of the head was 16½ inches and of the chest, 13 inches. The fontanels were wide open; the anterior measured 4 in. × 4 in. and the posterior, 2 in. × 2 in. The hair of the scalp and eyebrows was sparse and thin. The facies had a cretinoid appearance with a saddle nose, but the tongue was not enlarged or protruding. There were no teeth. The nipples were minute. There was a soft apical murmur over the precordium and the heart tones were muffled. The liver and spleen were not enlarged. The external genitalia were very small. The nails appeared atrophic and did not reach the end of the digits. The subcutaneous tissue was doughy and there was puffiness over the dorsum of the hands and feet. Aside from very sluggish reflexes there were no abnormal neurologic findings. She was not able to hold up her head or sit with support. She made no attempt to grasp objects. There was marked hypochromic microcytic anemia. The blood glucose level was 18 mg. per cent. No carpal bones were present, as shown by a roentgen study of the wrist, and the epiphyses of the femoral heads were absent (the latter normally appear at 5 to 8 months of age). The distal femoral epiphysis and proximal tibial epiphysis, which are usually present at birth, were present and well developed. The level of total serum cholesterol was 303 mg. per cent, and of total carotenoids, 297 I.U. per cent. The temperature ranged between 96.4° and 97.4° F.

Subsequent data during hospital and Out-Patient visits are illustrated in Figure 1. At the age of 2 years and 7 months she was hospitalized because of recurrent convulsions, weakness, anorexia and constipation. She appeared very pale, icteric and clammy, and barely responded to stimuli. The red blood count was 1,800,000 per cu. mm. and she received several blood transfusions. The blood glucose level was 17 mg. per cent. One week later, there was no uptake of administered radioactive iodine by the thyroid. Two weeks later, she had another severe convulsion which followed a short period of vomiting and diarrhea. The blood glucose level was 22 mg. per cent. At the age of 3 years she was again admitted because of severe anemia and required several blood transfusions. The general appearance was unchanged but she was now able to hold up her head and grasp at shiny objects.

At the age of 5½ years she was hospitalized for further study to determine the effect of ACTH and cortisone on the concentration of blood sugar and the tolerance for thyroid. Since the age of 18 months she had gained only 1½ pounds and had grown only 2 inches! The dentition was similarly retarded, so that she had only 6 upper and 6 lower deciduous teeth. The total serum cholesterol level ranged from 299 to 303 mg. per cent and the icterus index from 14 to 17 units. The values for blood glucose were 26 mg. per cent or below.

On admission (Fig. 2) at the age of 5½ years she weighed 13 pounds 8 ounces and was 24 inches long. The upper segment (vertex to symphysis) was 14½ inches and the lower

(symphysis to heel) was 9½ inches. The fontanels were open; the anterior measured 2 in. × 2 in. and the posterior, ½ in. × ½ in. The skin was pale, smooth, and had a sallow tint. The nipples were minute and the external genitalia were small but intact. The liver was ½ inch below the costal margin and the spleen was not palpable. "Dental survey revealed eruption of 12 primary teeth. These teeth appeared pearly in color. The erupted teeth were the 8 incisor and 4 first primary molars. There was no occlusion and the gum tissue overlay the occlusal surfaces of 2 molars. There was no apparent function. The eruption age was that of a 16 to 18 month old infant. There seemed to be gross underdevelopment of the maxilla in relation to the eruptive age. Roentgen exam revealed calcification of all primary teeth, enamel and dentin, with the exception of the distal third of the 2nd primary molars and cuspids. The calcification age in this patient was that of a 2½ to 3 year old. There appeared to be a lack of alveolar bone development in both the maxilla and the mandible." Roentgen examination of the long bones revealed no epiphyses in the wrist, hip or elbow. The bone age was that of a 3 to 4-month-old infant.

METHODS

The patients were hospitalized for study and subsequently observed in the Out-Patient department once or twice a week. During a control period (the first six days in the hospital) the following determinations were made: daily weight, pre-prandial and post-prandial blood glucose (2), hemoglobin, red and white blood cell counts (by usual methods), eosinophil counts after administration of ACTH (3) and of adrenalin (4), total serum cholesterol (5), sodium and potassium (6), chloride (7), total protein, albumin and globulin (8), non-protein nitrogen (9), calcium (10), phosphorus (11), alkaline phosphatase (12) and total carotenoids (13). The oral glucose-tolerance test was carried out with 1.75 Gm. of glucose per Kg. of body weight and blood samples were collected, fasting, and then every half hour for three hours. The intravenous glucose-tolerance test was performed with 0.5 Gm. of glucose per Kg. of body weight, given as a 20 per cent solution over a period of thirty minutes; blood samples were obtained as in the oral test. The epinephrine glucose-tolerance test was performed with 0.3 cc. of 1 in 1,000 fresh epinephrine solution given subcutaneously and blood samples were collected, fasting, and at ten, twenty, thirty and sixty minutes.

On the seventh hospital day, ACTH[1] (10 mg.) was given intramuscularly and every six hours for the following four days. The dose was gradually decreased to 2½ mg. by the sixteenth day. During this period the following determinations were made: pre-prandial and post-prandial blood glucose, daily weight, urinary reducing substance and ketones, direct eosinophil count (14), total serum cholesterol, sodium, potassium and chloride. The epinephrine glucose-tolerance test was repeated after eight days of ACTH therapy. From the sixteenth to the fifty-sixth day, ACTH was given at irregular intervals of from three to seven days in order to determine the

[1] Corticotropin Solution (Wilson), 40 USP units per cc., Lots 83530–34.

response of the blood glucose level to such interrupted dosages. Thyroid therapy (oral) was begun on the twentieth day, starting with 1/16 grain daily and increasing rapidly to 1½ grains in Patient L. L., and remaining at 1 grain daily in Patient J. L. Data on the daily weight, fasting blood glucose, total serum cholesterol and direct eosinophil count were obtained during this period. Following discharge from the hospital, the patients were observed once or twice weekly. Observations for fifteen weeks are included in this study. L. L. was given ACTH, 20 mg. once weekly, and desiccated thyroid 1/16 grain daily. J. L. was treated similarly but could not tolerate it after the eighth week. Weekly determinations of weight, to-

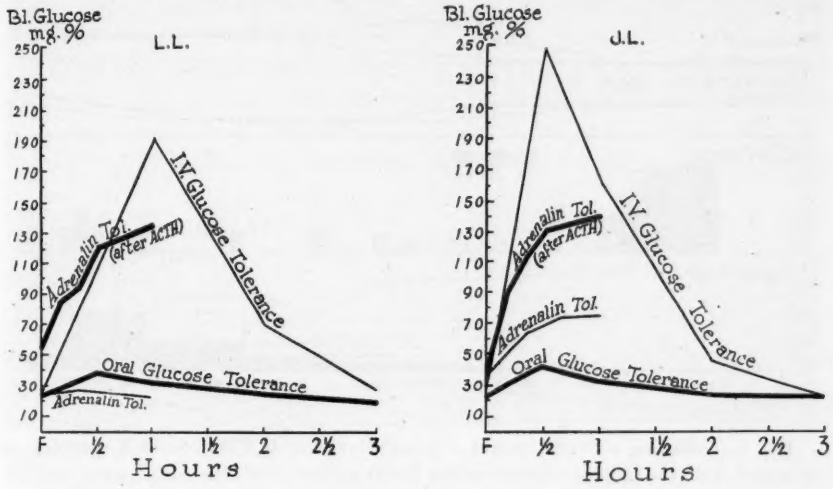

FIG. 3. Oral glucose-tolerance and epinephrine glucose-tolerance tests, illustrating unresponsiveness. Note adequate response with epinephrine seven days after ACTH therapy.

tal serum cholesterol, direct eosinophil count and fasting blood glucose were made and, at intervals, total serum protein, albumin and globulin, sodium, potassium and chloride. After the eighth week, ACTH and thyroid were omitted in J. L. and she was given cortisone[2] orally in daily doses, beginning with 25 mg. and decreasing to 2½ mg. The foregoing laboratory determinations were repeated.

RESULTS

Control period

The levels of fasting blood glucose ranged from 21 to 31 mg. per cent and of total serum cholesterol from 300 to 393 mg. per cent. The values for

[2] Cortone Tablets (Cortisone Acetate Merck), Lots 50802–04.

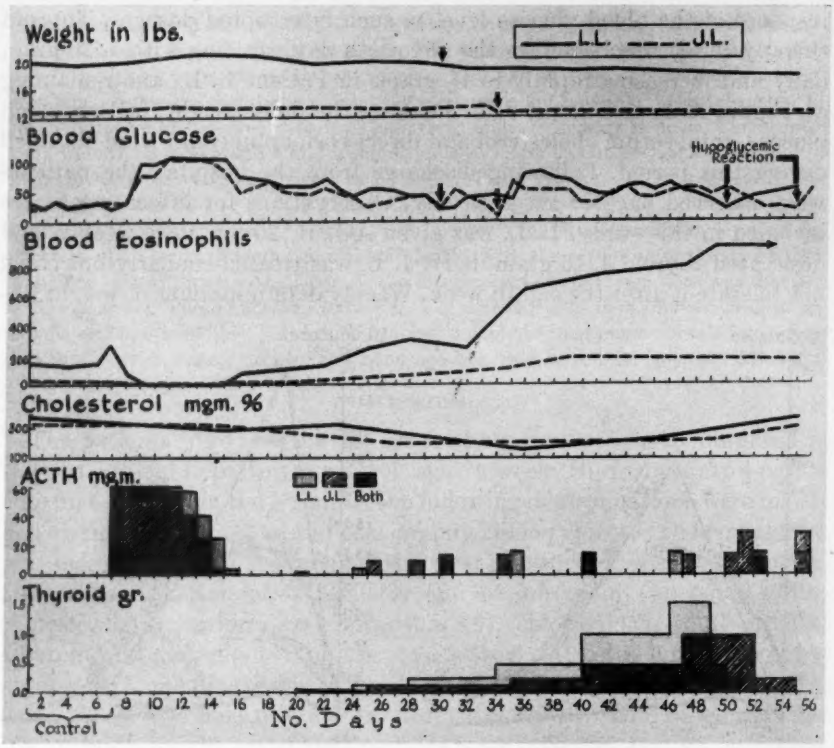

FIG. 4. Following a control period of seven days, and ACTH given daily in doses as indicated, note the prompt response of the blood glucose level, the eosinopenia, and the relatively unchanged serum cholesterol level. Interrupted therapy with ACTH was sufficient to "buffer" against the effect of increasing daily doses of thyroid. Short arrows indicate sharp drops in blood glucose levels without ensuing hypoglycemic symptoms. Hypoglycemic reactions are indicated by tall arrows following the cumulative effect of rapidly increased thyroid dosage.

serum sodium, potassium, chloride, total protein, albumin and globulin were normal. The results of cephalin flocculation tests for liver function were normal. ACTH (10 mg.) and epinephrine (0.3 cc. of 1 in 1,000 solution) produced an eosinopenia greater than 60 per cent at the end of four hours. The oral glucose-tolerance test gave a flat curve. The intravenous glucose-tolerance test showed a high peak at thirty and at sixty minutes, but a sharp drop to fasting levels at one hundred and eighty minutes. The epinephrine glucose-tolerance curve was relatively flat, with a slight rise at the end of sixty minutes (Fig. 3).

ACTH—daily administration

The response to ACTH was prompt and sustained (Fig. 4). Fasting

blood glucose levels ranged from 80 to 115 mg. per cent during ACTH administration. There was a noticeable increase in appetite and intake of food and a progressive gain in weight (values for serum sodium and chloride remained unchanged). There was moderate glycosuria for two days but no ketonuria. Of decided interest was the change in the epinephrine glucose-tolerance curve at the end of eight days of ACTH therapy—blood glucose values of 133 to 141 mg. per cent, respectively, in both siblings after sixty minutes (Fig. 3). The eosinopenia, which occurred after the first dose of ACTH, remained throughout the whole period of therapy. Serum cholesterol values were relatively unchanged.

ACTH at intervals and desiccated thyroid daily

ACTH was given in doses of from 2.5 to 15 mg. every four or five days, when the value for blood glucose tended to fall below the average of the preceding three days, or when anorexia and fever intervened (Fig. 4). Fasting values for blood glucose were maintained satisfactorily with inter-

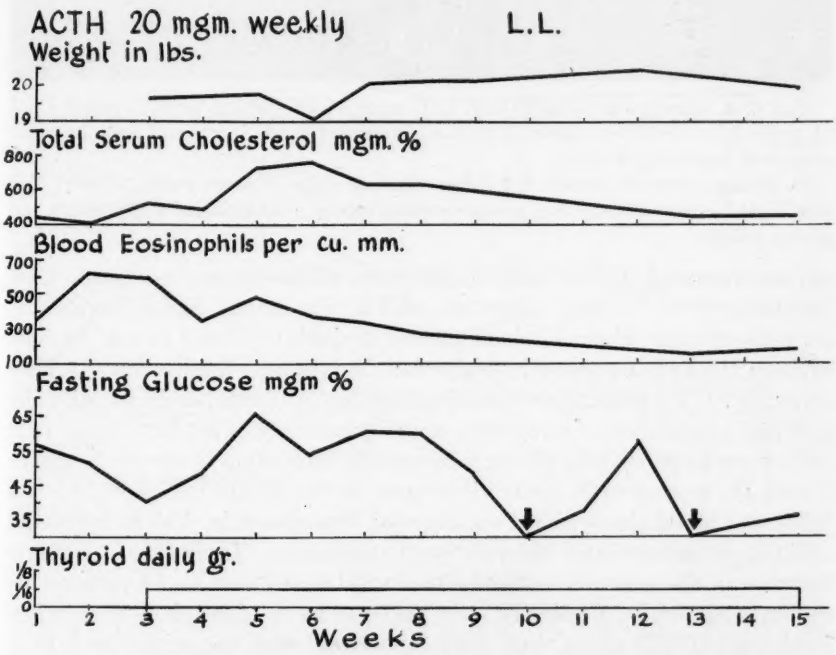

FIG. 5. Following the hypoglycemic reaction (in L. L.) as illustrated in Figure 4, thyroid was discontinued for three weeks and ACTH was administered in a dose of 20 mg. weekly. Resumption of thyroid was followed by a fall in eosinophils and in the serum cholesterol level. Blood glucose levels were maintained satisfactorily without hypoglycemic symptoms, despite sharp drops, as indicated by arrows.

FIG. 6. *A.* Changes noted in Patient L. L. after ACTH (20 mg. weekly) and thyroid ($\frac{1}{16}$ grain daily) for fifteen weeks. Note loss of cretinoid facies, increase of scalp hair and absence of protruding tongue.

B. Changes noted in Patient J. L. after cortisone daily for seven weeks, without thyroid. Note increase in scalp hair, greater responsiveness, change in facial expression and gain in weight.

mittent doses of ACTH and ranged from 22 to 86 mg. per cent. This "protective" or "buffer" effect of ACTH was noted during periods of anorexia or fever, when the blood glucose dropped to 17 and 18 mg. per cent without the appearance of hypoglycemic symptoms. Again, this "buffer" action of ACTH permitted the administration of thyroid daily without the striking hypoglycemic symptoms noted previously. Following from four to five weeks of thyroid therapy in rapidly increasing doses up to grains 1 and $1\frac{1}{2}$, respectively, there developed an accumulative effect, with a fall in the blood glucose level and signs of hypoglycemia. Values for serum sodium, potassium and chloride were unchanged. There was a definite decrease in the concentration of serum total cholesterol and a progressive rise in eosinophils. Clinically, the patients were more alert than when receiving ACTH alone, but their appetites were capricious and they lost weight.

L. L.—ACTH (20 mg. weekly) and thyroid (1/16 grain daily)

ACTH in this dose was sufficient to "buffer" against the hypoglycemic effect of a daily dose of thyroid for fifteen weeks (Fig. 5). At the tenth and

thirteenth weeks, blood glucose levels decreased to 28 and 30 mg. per cent, respectively, without the onset of hypoglycemic symptoms. With resumption of thyroid after discharge from the hospital, the total eosinophils again decreased and the serum cholesterol level fell slowly. The appetite improved and the weight increased. Scalp hair continued to grow perceptibly and the tongue was smaller and no longer protruded (Fig. 6A). The epiphyses of the wrist showed greater density and size and a small epiphysis appeared at the head of the radius. However, there was no increase in bone length.

J.L.—Cortisone (daily) without thyroid

Daily cortisone produced a sustained elevation of the blood glucose level, increased appetite and a gain in weight (Fig. 7). Values for serum sodium, potassium and chloride were not altered. There was a sharp drop

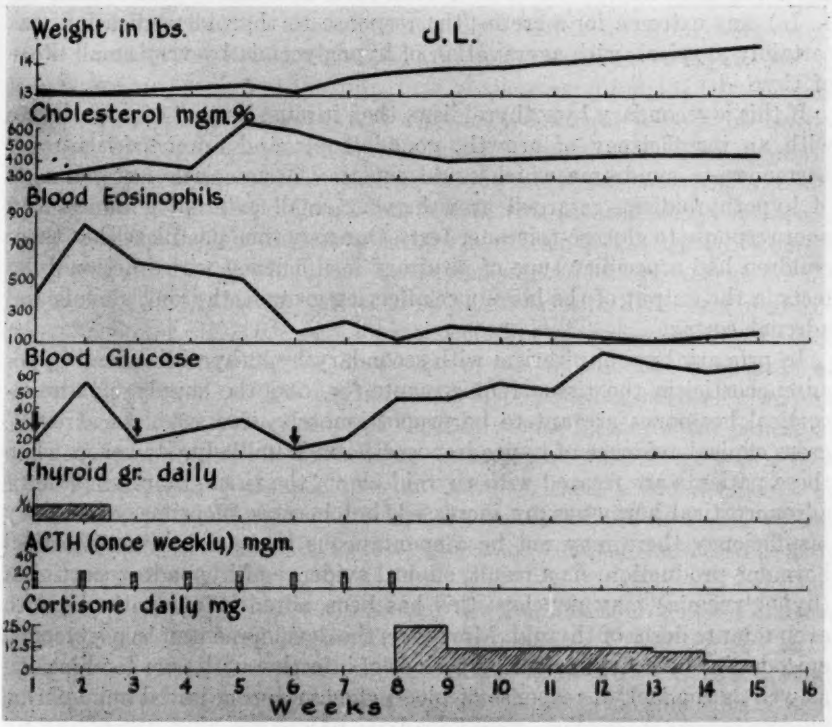

FIG. 7. Arrows indicate severe hypoglycemic reactions in Patient J. L. with ACTH and thyroid, as well as with ACTH alone. Cortisone given daily as indicated produced a progressive increase in the blood glucose level, which was maintained between 40 and 60 mg. per cent with as little as 2.5 mg. of cortisone daily. The increase in weight was associated with greater activity and intake of food.

in eosinophils but little change in the serum cholesterol level. Clinically, the patient appeared more active, the facies became less cretinoid and the hair of the scalp increased in length and thickness (Fig. 6B). The long bones showed no change in maturation or length.

DISCUSSION

It is evident that these children were hypothyroid, as exhibited by the retardation of growth and development, the absence of radioiodine uptake by the thyroid, the cretinoid facies and body habitus, hypothermia, large tongue (in L. L.), elevated levels of serum cholesterol and carotenoids, and flat glucose-tolerance curves. However, there are other features which indicate that these were not examples of a primary athyrosis. Although the features were cretinoid, they were not typically those of a cretin; the skin was warm and velvety instead of dry and coarse; the growth and development were too consistently retarded and the hypogonadism (in L. L.) was extreme for a cretin; the response to thyroid medication was certainly atypical, with aggravation of hypoglycemia by very small doses of thyroid.

If this is secondary hypothyroidism, then it must be part of a syndrome with an insufficiency of growth, gonadotropic and adrenocorticotropic hormones—a syndrome which could explain the association of elements of hypothyroidism, retarded growth, very small external genitalia and poor response to glucose-tolerance tests. One may then postulate that these children had a peculiar type of pituitary insufficiency with fractional defects in the output of the hormones affecting growth, thyroid, gonads and adrenal cortex.

In primary hypopituitarism with secondary hypothyroidism and hypoadrenocorticism the tissue requirements for, and the supply of adrenocortical hormones are apt to be proportionately decreased. As a result, gross clinical evidence of hypoadrenocorticism is unlikely. However, when these patients are treated with thyroid alone, the tissue requirements for adrenocortical hormones are increased, but because of primary pituitary insufficiency there may not be a spontaneous increase in adrenocortical hormone production. As a result, clinical evidence of hypoadrenocorticism (hypoglycemia) may develop; this has been noted in our patients, with even minute doses of thyroid. Moreover, the development of hypoglycemic episodes in these siblings during periods of infection with poor food intake, as well as the lack of response of blood glucose during initial epinephrine glucose-tolerance studies, would lead us to assume that there was an insufficient supply of liver glycogen resulting in part from deficiency of adrenocortical hormones. In contrast, patients with primary hypothyroidism are apt to have secondary hypopituitarism and adrenocorticism

and may resemble patients with pituitary insufficiency. However, when thyroid is administered, signs of hypoadrenocorticism do not develop because the pituitary and adrenocortical functions are restored to normal with thyroid therapy. Means, Hertz and Lerman (15) and, later, Lerman and Stebbin (16) described a "pituitary type of myxedema" in older patients who did not respond favorably to thyroid and in whom hypoglycemia and adrenocortical insufficiency developed. At autopsy (17), the endocrine glands showed the characteristic changes of Simmonds' disease, with atrophy of the pituitary, thyroid, adrenals and gonads.

Concerning the etiology of hypoglycemia per se, absolute (or true) hyperinsulinism with overproduction of insulin by the beta cells of the islets of Langerhans is considered. Tumors of the islets of Langerhans are rare in children, and although hyperplasia of the islets does occur, it is not a common cause of hypoglycemia (19). The associated endocrinopathy in our patients would tend to exclude such a possibility. Relative hyperinsulinism refers to the hypoglycemia which results, not from overproduction of insulin but from underactivity of one or more of the organ systems which are antagonistic to insulin. One of these conditions is hypoadrenocorticism (Addison's disease) and a primary defect of the adrenal cortex can be ruled out on the basis of clinical findings, as well as upon the fact that the disturbance of carbohydrate metabolism was not typically Addisonian (20). As previously suggested, this associated hypoglycemia may be on the basis of anterior pituitary insufficiency (particularly a defect in growth hormone, which has an anti-insulin effect) as well as on the basis of a defect in adrenocorticotropic hormone with deficiency of gluconeogenic factor (21). Other etiologic aspects, such as extensive liver disease or glycogen storage disease of the liver, can be eliminated because of negative findings. Another mechanism for hypoglycemia has been proposed by McQuarrie (18), the absence of "hyperglycemic factor" of the pancreas, secreted by the alpha cells of the islets. In his report of 2 siblings with severe hypoglycemosis and convulsions since infancy, there was a deficiency in the number of alpha cells and a loss of granules of the beta cells of the islets. However, notable endocrine anomalies were not associated with this type of hypoglycemia.

The use of ACTH and cortisone for the modification of blood glucose concentration has been justified by the experience of many others, ever since the work of Carey (22) and Long (23) demonstrated that adrenal cortical extract in excessive doses exerted a diabetogenic effect in the normal animal. The work of Houssay (24), Evans (25), Baumann (26) and Young (27) indicated that continued administration of extracts of the anterior lobe of the pituitary produced glycosuria and hyperglycemia in the normal animal. In our patients, daily doses of ACTH and cortisone

effectively elevated and maintained blood glucose levels. The response to interrupted doses of ACTH, although less sustained, was still sufficient to keep the level of blood glucose above average control values. This response was, however, influenced by concomitant thyroid therapy. It is notable that even such interrupted doses of ACTH seemed to "buffer" the blood glucose level against precipitous falls with hypoglycemic symptoms, as noted previously. Apparently, even such "buffer" action of ACTH was ineffective against the cumulative action of rapidly increased doses of thyroid over a period of from four to five weeks.

A comparison of the blood glucose responses during epinephrine glucose-tolerance studies would seem to indicate a more adequate glycogen reserve in the liver following ACTH therapy. The response of the serum cholesterol level to ACTH and cortisone was variable and was probably modified by the concomitant use of thyroid. Hypercholesterolemia has been reported by Wolfson (28), Adlersberg (29) and Fredrickson (30). Barson and Yalow (31) found no consistent changes in the concentration of serum cholesterol during administration of cortisone. During intensive therapy with ACTH alone for seven days (in L. L. and J. L.) and daily cortisone for eight weeks (in J. L.) there was little change in the serum cholesterol levels. However, when daily thyroid was added to the intermittent doses of ACTH, there was a gradual fall in the level of serum cholesterol, followed by a prompt and sustained rise after thyroid was discontinued. It is difficult to rationalize the prompt eosinopenic response to ACTH in these 2 patients with pituitary insufficiency, for it has been said that "prompt response to ACTH by a hypopituitary patient suggests that the pituitary disease is mild or of a very recent origin" (32). Similarly, the eosinopenic response to epinephrine is usually not encountered in hypopituitary patients unless we assume that epinephrine may cause lysis of circulating eosinophils (33). Furthermore, it is difficult to understand the rapid increase in eosinophils while these patients were receiving interrupted doses of ACTH and increasing amounts of thyroid, unless we assume the development of refractoriness to ACTH or some effect from large doses of thyroid. It is noted in Patient L. L., who later received weekly doses of ACTH and small daily doses of thyroid, that the eosinophils decreased significantly. Similarly, in Patient J. L., the eosinophils remained at low levels with daily doses of cortisone.

The failure to obtain growth in these siblings may emphasize the fact that the thyroid hormone does not stimulate growth in the absence of the pituitary growth hormone (34 a, b, c). There may be other factors influencing this action, namely, the amount of thyroid used, the duration of therapy and the concomitant therapy with ACTH.

It is impossible at this time to develop a unitary etiology for the unusual

syndrome in these siblings. Definite evidence exists for thyroid deficiency, adrenocorticotropic deficiency, gonadal underdevelopment and retarded skeletal growth. This can best be summed up as a peculiar type of pituitary insufficiency. In addition, the underdevelopment of cerebral functions, usually not associated with hypopituitarism, may be either primary to the whole syndrome or the result of chronic hypoglycemia and lack of thyroid.

SUMMARY AND CONCLUSIONS

1. The association of chronic hypoglycemia and convulsions with extreme dwarfism is reported in 2 siblings who showed marked sensitivity to oral thyroid.

2. The results of therapy with ACTH and cortisone as well as ACTH and thyroid are reported. The effect of this therapy upon blood glucose and cholesterol levels, eosinophils, weight, growth and general clinical condition is discussed.

3. ACTH or cortisone alone effectively elevated and maintained blood glucose levels. Interrupted doses of ACTH, despite increasing daily amounts of thyroid, were sufficient to "buffer" the blood glucose level against precipitous falls, except for the cumulative effect of large doses of thyroid.

4. Neither ACTH nor cortisone markedly altered the serum cholesterol level.

5. Eosinopenia developed during ACTH and cortisone therapy; the increase in total eosinophils during ACTH-thyroid therapy is discussed.

6. Definite weight loss followed prolonged ACTH-thyroid therapy.

7. It is postulated that the unusual dwarfism of these siblings is the result of an insufficiency of the anterior pituitary, perhaps secondary to a congenital defect of the hypothalamus, with fractional deficiencies of hormones concerned with growth, the thyroid, gonads and adrenal cortex; and that the hypoglycemia is the result of hypofunction of one or more of these "target" organs.

Acknowledgment

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DESOXYCORTICOSTERONE IN RABBITS: SIMULATION OF RABBIT "TOXEMIA OF PREGNANCY"*

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EFFECTS of prolonged administration of desoxycorticosterone acetate (DCA) have been amply documented in rats, chicks, dogs and man (1). Observations in rabbits are scanty or lacking in detail. In the rat DCA, under appropriate conditions, elicits arterial hypertension and hypertensive vascular disease; in the dog it provokes neither substantial hypertension nor hypertensive disease, although it alters the metabolisms of water, sodium and potassium, and causes paralysis from lack of potassium. In human beings sensitized to DCA by severe adrenal or, as seems likely, renal disease, it elicits edema, hypertension, and occasionally vascular lesions (2, 3).

The few experiments in rabbits have been oriented towards production of a state similar to eclamptogenic toxemia of human beings and have not been entirely rewarding. Thus, Dexter and Weiss (4) found no substantial effect of DCA at the end of two weeks; Rakoff, Paschkis and Cantarow (5) noted no ill effects in normal rabbits after two to three months, although their 2 pregnant rabbits weakened, aborted and died during treatment. Verniory (6) noted that some normal rabbits treated over several weeks died and manifested edema and paralysis; he was unable to demonstrate any definite effect of DCA on arterial pressure. More recently, Parviainen, Soiva and Ehrnrooth (7) reported that 3 rabbits given DCA and salt showed mild "nephrotic" renal changes and small foci of hepatic necrosis. In the experiments cited, the steroid was injected in oil solution, a form in which the steroid is not as effective as in water suspension or pellet implant. The data include observations on survival, arterial pressure, muscular strength and pregnancy. There were suggestive renal and hepatic lesions.

Our interest in the effects of DCA on rabbits arose from the syndrome elicited by administration of renin-containing renal extracts to rats pretreated with DCA (8); it is characterized by edema, oliguria and convul-

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sions; the lesions include degenerative deposits in glomerular tufts and colloid-like thrombi in the small vessels of kidney, brain and other organs (9). This condition differs from human eclampsia principally in the absence of hepatic lesions. It was considered that it might result from specific resistance of the rat to the hepatic effects of DCA or of DCA+renin. Further, the rabbit is known to be susceptible to a spontaneous "toxemia of pregnancy," one of the major characteristics of which is hepatic damage (10).

For these reasons, studies were initiated on the effects in salt-treated rabbits of prolonged subcutaneous administration of DCA in water suspension.

METHODS

Rabbits of the New Zealand white strain were divided into two groups evenly distributed as to sex: 22 were given DCA (group I) and 10 were kept as controls (group II). Subsequently, 6 rabbits of mixed strain (group III) were subjected to the same treatment as was group I. The treatment period, unless terminated by death of the animal, was twenty days; at the end of this period, survivors were killed and autopsied. The diet in each group consisted of commercial rabbit pellets[1] and of 1 per cent sodium chloride as a substitute for water. Treatment with sodium chloride was begun one week before injection of DCA. The injections of DCA were made daily in groups I and III in doses of 10 mg. suspended in 1 cc. of a mixture of water, alcohol and detergent; 1-cc. doses of steroid-free medium were administered daily in group II. In group I, 4 animals, and in group II, 3 animals were known to be pregnant at the beginning of treatment.

Blood was sampled before, during and at the end of the experimental period. The analyses included hemoglobin, hematocrit, serum sodium and potassium (by flame photometry), serum cholesterol (11), and electrophoretic (12) and ultracentrifugal (13) fractionations of plasma or serum proteins. In some of the animals, arterial pressures, electrocardiograms and electroencephalograms were recorded during the experiment.

The organs of animals dying during the experiment or killed at its end were examined grossly; selected tissues were fixed in Zenker's fluid or in 10 per cent formalin for microscopic study; the stains used on tissue sections were hematoxylin-eosin-methylene blue, periodic acid-fuchsin, trichrome and Sudan IV.

RESULTS

A. *Clinical course.* In most (20 of 22) of the steroid-treated animals in group I severe motor disturbances developed; the onset of these was abrupt, sometimes as early as the fifth, but commonly about the twelfth day. They became manifest as weakness and paralysis or as tetanic seizures with motor hyperirritability; the disturbances were sometimes concomitant. Weakness and paralysis appeared first in the muscles of the neck and shoulder and progressed in extent and severity to the degree that some of the animals drowned in their drinking cups. Hyperirritability

[1] Broadview Feed and Seed Company, Cleveland, Ohio.

was manifested initially in abrupt withdrawal when approached or lightly touched; this progressed to spasm of the muscles of the shoulder and forelimbs and tetanic movements of the hind limbs and head. The disturbances seemed to be independent, and appeared in no distinct sequence. Most of the animals manifesting either disturbance died in from three to four days after its onset. Possibly because of technical difficulties (muscle waves in awake rabbits, and effects of pentobarbital in anesthetized rabbits), electroencephalograms recorded during the phases of motor disturbances did not show any definite abnormality.

Pregnant animals in group II continued their pregnancies without incident. In contrast, the 4 DCA-treated pregnant animals in group I did not deliver spontaneously and at autopsy were found to have retained macerated fetuses.

Arterial pressures were measured from foreleg pulse, by an adaptation of the method of Olmsted, Corcoran and Page (14), in animals of both groups I and II and in some of these, under anesthesia. No increase of arterial pressure was observed during treatment (group I, mean 67, range 55 to 77 mm. Hg; group II, mean 91, range 68 to 115).

The 1 per cent sodium chloride ingested was the only fluid intake; this averaged about 100 cc. daily in group II; it increased to about 700 cc. daily in group I, only to decrease sharply to 50 or 100 cc. with the onset of motor disturbances.

The 6 animals of mixed strain (group III) treated with DCA survived the experimental period and showed none of the motor disturbances which characterized the identically treated New Zealand whites of group I. At autopsy, they showed no gross lesions.

B. *Blood chemistry.* Most of the observations are summarized in Table 1. Hemoglobin, hematocrit, serum chloride and sodium were not significantly altered by DCA (group I). Serum potassium concentration was decreased in paralytic and tetanic animals; the hypokaliemia did not result

TABLE 1. BLOOD CHANGES IN THE RABBIT FOLLOWING TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

Treatment	No. of animals	Hematocrit ratio	Hemoglobin (Gm. %)	Serum Cl (mEq./L)	Serum Na (mEq./L)	Serum K (mEq./L)
DCA	12	.34 (.26–.44)	10.3 (7.7–12.6)	112.9 (101–118)	143 (139–150)	2.1 (1.3–3.3)
—	8	.35 (.25–.42)	10.3 (8.5–10.9)	110.5 (95–120)	141 (134.5–147)	5.2 (3.8–6)

TABLE 2. EFFECTS IN RABBITS OF DESOXYCORTICOSTERONE ACETATE ON SERUM LIPOPROTEIN PATTERN AND CHOLESTEROL

Group and animal No.	$S_{1.21}$ in mg. per 100 ml.				Cholesterol (mg. per 100 ml.)	Remarks
	>70	40–70	35	6		
I. DCA						
464-2	142	62	95	21	111	Neck paralyzed. Hyperexcitability.
464-4	178	43	47	28	72	Neck paralyzed.
491-1	71	36	24	47	149	Back paralyzed. Pleuritis.
491-5	178	71	7	<7	98	General paralysis.
491-2		28	83	71	61	Hyperexcitability.
464-3	237	118	36	36	125	Paralysis. Fetuses dead.
Average	161	60	48	34	102	
II. CONTROL						
506-2			17	71	21	
492-2			12	19	22	
492-1			36	26	41	
491-3			43	90	44	
491-4			28	31	—	
463-4			12	71	16	
463-2			33	38	30	
492-4			14	43	22	
491-5			24	33	23	
491-2		12	31	66	52	
491-1			31	62	55	
463-3			12	36	83	Pregnant.
463-1	9	14	47	71	—	Post partum.
Average	1	2	26	51	37	

in depression of T waves in the electrocardiograms recorded during the paralytic phase.

The measurements of serum cholesterol and lipoproteins were undertaken because the sera of treated animals were found to be lipemic. As seen in Table 2, the visible lipemia of the DCA-treated rabbits was found to be associated with hypercholesterolemia. Lipoproteins[2] were measured by flotation analysis after ultracentrifugation in a medium of density 1.21, which permits simultaneous estimates of α and β fractions (Table 2). Normal rabbit sera showed under these conditions, two components: one, with a flotation rate of −S35, occurring in a mean concentration of

[2] "+S" = 1 Svedberg sedimentation unit = 10^{-13} cm./sec./dyne/Gm. "−S" identifies flotation in a medium of density 1.21.

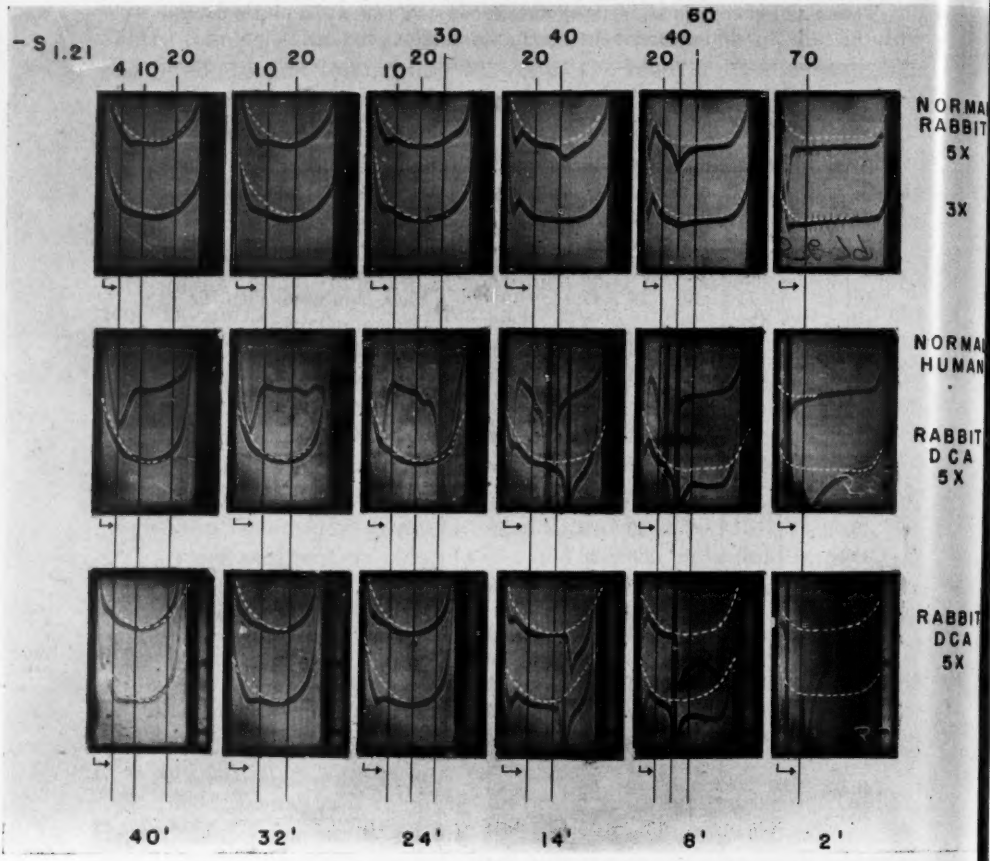

FIG. 1. Lipoprotein patterns of normal rabbit (above), normal human and normal rabbit (middle) and DCA-treated rabbit (below). The concentration factor of the serum lipoprotein is indicated as X at the right margin. The numerals above indicate rates of negative sedimentation (flotation) at a density of 1.21 and a temperature of 26° C.

26, and the other, with a flotation rate of −S6, in concentrations averaging 51 mg. per 100 cc. Analyses made after the onset of motor disturbances in group I revealed components with more rapid rates of flotation (−S >70 and −S(40–70)) in mean concentrations of 161 and 60 mg. per 100 cc., respectively; the concentration of the −S35 component was slightly increased (48 mg. per 100 cc.) and that of the −S6 fraction slightly decreased (34 mg. per 100 cc.). The animal (464–3) with the most abnormal lipoprotein pattern was pregnant at the time of sampling, and at autopsy (two days later) the fetuses were found to be macerated. Small, but meas-

urable concentrations of the abnormal components $-S>70$ and $-S(40-70)$ occurred in the serum of 1 control rabbit (463–1) immediately post partum. Representative liproprotein patterns are shown in Figure 1.

The electrophoretic analyses were carried out in phosphate buffer pH 7.8, ionic strength 0.16μ. In terms of the mean composition of the total protein, the fractions found in normal female rabbits were: albumin, 67; α_2-globulin, 5; β-globulin, 12; ϕ-globulin (fibrinogen), 6; and γ-globulin, 10 per cent. Animals treated with DCA showed decreases in albumin, separation of α_1-globulin (not resolved from albumin in normal sera under these conditions), slight increases in concentration of β-globulin and fibrinogen and a marked increase in γ-globulin. Concentrations of these fractions in group I were: albumin, 49; α_1-globulin, 12; α_2-globulin, 9; β-globulin, 14; γ-globulin, 5; and fibrinogen, 11 per cent of the total proteins. The patterns found in groups I and II were respectively uniform; they are depicted in Figure 2.

C. *Morphologic changes.* Neither gross nor microscopic lesions were found in animals of the control group. Effusions were observed in the serous cavities of some animals of group I, although none showed edema of subcutaneous tissues or viscera. In general, the lesions of those which survived were similar in kind, although less severe, than those found in animals which had succumbed during treatment. The organs which showed gross changes were liver, kidney and heart. The livers were friable, congested or yellowish, with an exaggerated lobular pattern. In most of the animals, areas subsequently shown to be necrotic were found; these were often overlain by subcapsular hemorrhages; they were more frequent and sometimes exclusively present in the periphery of the right lobe. That coccidiosis was not the cause of these lesions was indicated by their distribution and by the fact that they did not occur in control animals. The kidneys of treated animals were pale; streaks of intense pallor were noted in the cortical zone; wedge-shaped areas of sclerosis with pitting of the surface were found in 2 of the treated animals. The gross cardiac lesions consisted of occasional subendocardial hemorrhages.

On microscopic examination, the hepatic lesions varied widely in severity. The less severe consisted in dilatation of sinusoids around the centrolobular veins and fatty vacuolization of adjacent cells (Fig. 3 a). Section of livers of control animals stained with Sudan IV showed fine particles of fat evenly distributed throughout the parenchyma, whereas similar sections from treated animals showed aggregation of the fat in irregularly distributed globules with some tendency to concentration in the mid-zone of the lobule. The more severe changes, characteristically predominant in the right lobe of the liver, consisted in necrosis (Fig. 3 b); in some sections large continuous areas of necrosis occurred, in which only

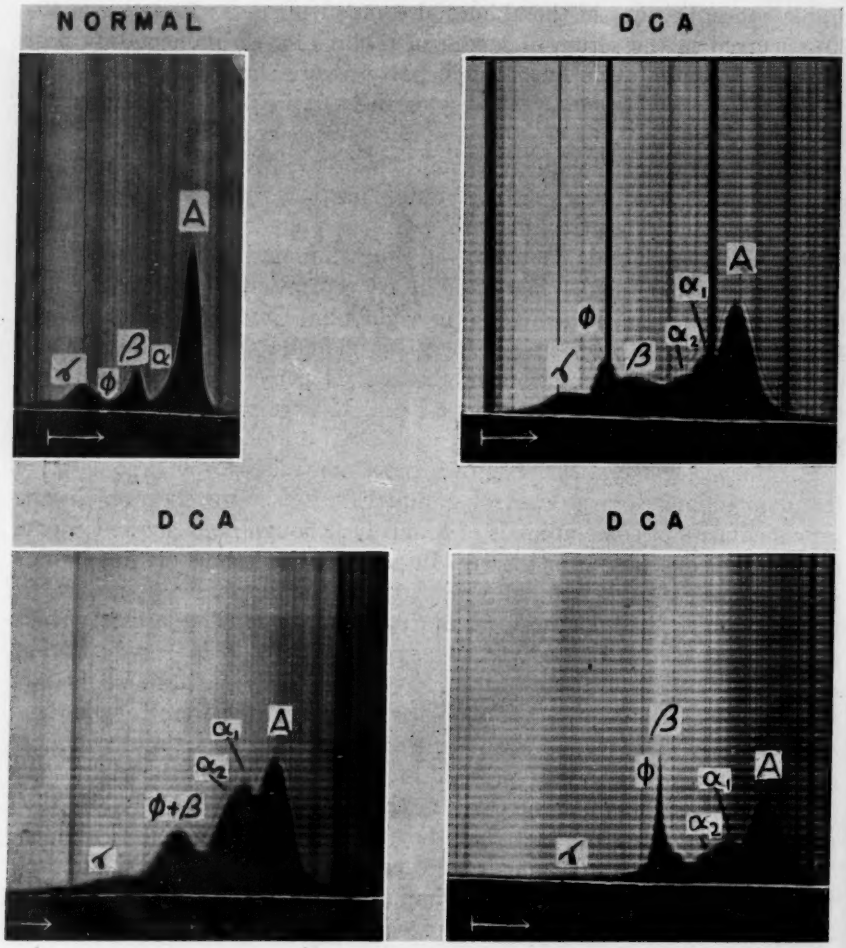

FIG. 2. Electrophoretic patterns of plasma proteins in phosphate buffer pH 7.8, ionic strength 0.1, of normal and DCA-treated rabbits.

the portal spaces remained relatively intact; in other sections the necrosis involved only the mid-zonal regions of the lobules (Fig. 3 c). The necrotic lesions of animals surviving the experiment were invaded by neutrophils; the portal spaces, including those not involved in necrosis, were often surrounded by cuffs of mononuclear cells (Fig. 3 d). The grossly visible subcapsular hemorrhages were found to occur only over foci of necrosis. No fibrin thrombi were demonstrable.

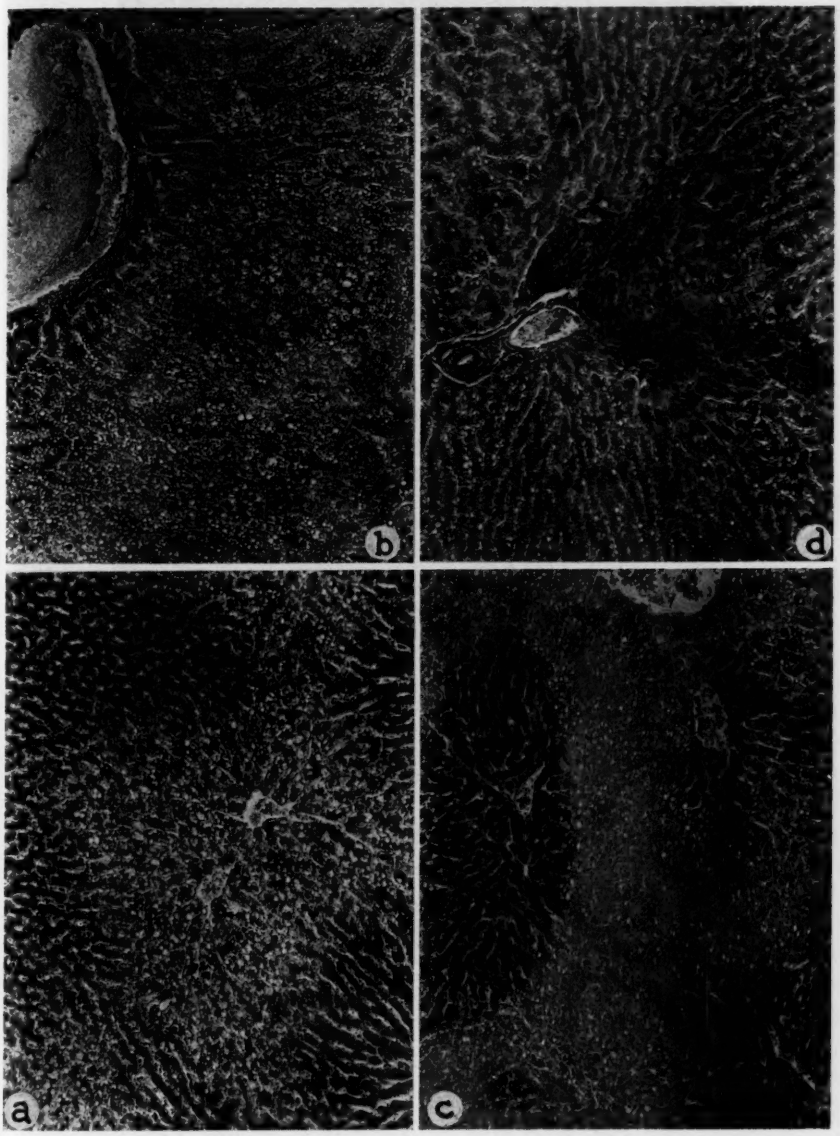

FIG. 3. Hepatic lesions of varying severity as described in text. Hematoxylin-eosin-methylene blue (X 70).

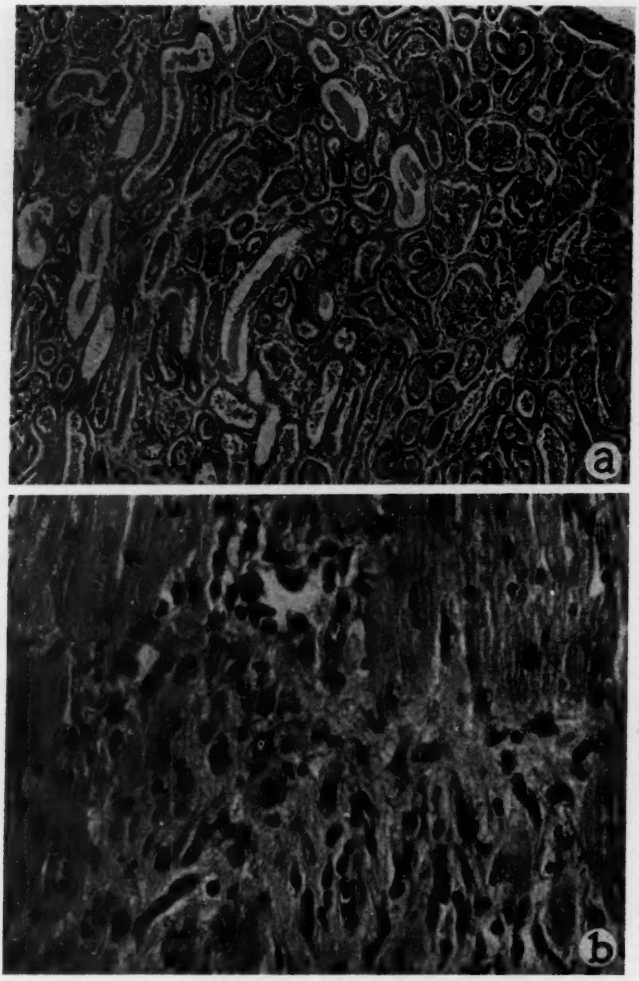

FIG. 4. (a) Renal and (b) myocardial lesions, as described in text. Hematoxylin-eosin methylene blue (X 70 and X 200).

The common renal lesions were interstitial edema, tubular dilatation, and appearance of finely granular and hyaline casts; the latter changes were sometimes very severe (Fig. 4 a). Glomeruli were enlarged and capsular spaces contained a finely granular precipitate. Areas of cortical necrosis were demonstrable in 7 of the DCA-treated animals; wedges of well-preserved cortex separated the areas of necrosis; the medulla was relatively intact and never necrotic. No degenerative accumulations of

polysaccharide-containing material and no thickening of the basement membrane were demonstrable by periodic-acid fuchsin and Lee-Brown stains.

Abnormalities which may well have been incidental were (a) cellular infiltration of occasional glomeruli, (b) occasional thickening of capsular membranes and (c), as noted above, wedge-shaped areas of sclerosis with atrophy or dilatation of tubules in the affected areas, but without evident vascular lesions.

Microscopy of the myocardium showed most commonly, focal myocarditis which was principally subendocardial. In such lesions the degenerated muscle fibers were replaced by fibroblasts, Anitschkow myocytes, and some mononuclear cells (Fig. 4 b); no giant cells or other elements resembling those seen in experimental periarteritis nodosa (15) could be identified.

DISCUSSION

1. *Species and DCA actions*

It is a familiar observation that species is an important determinant of hormonal action. The effects of DCA admirably illustrate this principle, since, from the several aspects of clinical course, blood chemistry and morphologic changes, its effects in the rabbit differ significantly from those observed in rat, dog and man.

(a) *Clinical course.* Rabbits treated with DCA and sodium chloride show the development of polyuria, weakness and/or paralysis—with a curare-like sequence—hyperexcitability, and tetanic seizures; they do not have hypertension or hypertensive arterial disease; they do demonstrate death and retention of fetuses and commonly death of the experimental subject. Of these phenomena, polyuria is also observed in both rats and dogs; rats are extraordinarily resistant to DCA paralysis, which in dogs and rabbits is associated with hypokaliemia. In rats, severe potassium depletion is antihypertensive and is associated with loss of vascular responsiveness to pressor agents (16). The tendency to hypotension in DCA-treated rabbits can be explained on this basis. From this, one might conjecture that the resistance of rabbits and dogs to the hypertensive effects of DCA may be an aspect of their greater susceptibility to the paralytic-hypokaliemic effects of DCA. As concerns pregnancy, DCA is known to maintain pregnancy in ovariectomized rats and to cause abortion in normal animals when injections are discontinued (17); both effects can be attributed to the progestational action of DCA (18). The mechanism of fetal death and of death in the experimental subjects is obscure, although it seems likely that it follows in the wake of advancing paralysis of respiratory and/or cardiac muscles.

The convulsive phenomena observed in rabbits given DCA are not observed in rats or dogs, and, since they are often concurrent with muscle weakness or paralysis, they are somewhat paradoxical. DCA in rats increases the convulsive threshold to electroshock (19). The possibility that DCA may depress serum calcium was unfortunately not examined in these experiments. Hepatic necrosis is sometimes associated with convulsions and tetany and it may be that this association prevails in rabbits.

(b) *Blood chemisty.* The only change in electrolytes is depression of the serum potassium level, which is interpreted as a manifestation of increased potassium excretion and, in view of its association with paralysis, of a substantial deficit in intracellular potassium. Indeed, Parviainen, Soiva and Ehrnrooth (7) have found that administration of DCA to rabbits increases the tissue Na/K ratio, apparently by replacement of potassium by sodium. However, in rats, dogs and man such severe states of potassium deficiency are associated with hypochloremic alkalosis, in which serum chloride and carbon dioxide become, respectively, parallel and reciprocal functions of tissue potassium concentration (20, 21). This hypochloremia is interpreted as a sequel to potassium depletion and intracellular acidosis in the cells of the distal convoluted tubule (22). In this respect, the rabbit seems to be unique, since hypokaliemia is not associated with hypochloremia; anomalous also is absence of the hypernatremia commonly found in rats and dogs given large doses of DCA. Thus, it seems that rabbits differ from other species in their renal responses to DCA and to potassium depletion. Perhaps relevant in this regard is the observation that rabbits given DCA do not show increased rates of migration of sodium and chloride into intraperitoneal pools of 5 per cent glucose, whereas such increased rates are demonstrable in dogs (23).

The DCA-induced lipemia and the associated changes in serum cholesterol, lipoprotein and electrophoretic patterns seem to be unique. These are not known to occur in other species, so that it has been widely assumed that DCA has no effect on fat metabolism. However, cortisone is known to induce in rabbits (24), but not in man (25), changes in lipoprotein pattern qualitatively similar to those which result from DCA. Others (26) have noted that DCA does not influence exogenous hypercholesterolemia or atherogenésis in rabbits; however, the doses given were very small (5–7 mg. weekly). Rather, one might associate the effect of DCA on serum lipids in rabbits with the known susceptibility of this species to atherogenic lipid changes, were it not that in another susceptible species, the chick, DCA in relatively large dosage neither alters serum cholesterol nor changes the course of exogenous hypercholesterolemia (27). Another mechanism has been suggested to us: heparin is known to participate in the formation

of an antilipemic "plasma clearing factor" (28) and, since DCA is believed to suppress heparin action or heparin formation (29), it might be that the lipemia observed in rabbits is due to a deficiency of endogenous "plasma clearing factor."

Descriptively, the alterations in serum lipoproteins resemble those which result from feeding cholesterol or giving cortisone to rabbits although, as compared to cortisone, they are more intense. It can be assumed that the alterations in lipoproteins determine many of the changes induced in the electrophoretic patterns of the plasma proteins.

(c) *Morphologic changes.* From the foregoing, it appears that DCA elicits severe potassium deficiency in rabbits, which can be assumed to account for the few myocardial lesions observed. In contrast to other species, the DCA-treated rabbits manifest hepatic and renal necroses but, under the condition of the experiment, do not show hypertension, arteritis or other vascular lesions. The mechanism of the distinctive hepatic and renal lesions is not apparent. Similar hepatic lesions have been produced in this species by injections of thromboplastin (30), posterior and anterior pituitary extracts (31) and by placental perfusate (32). These lesions have been attributed to vascular occlusions, as demonstrated by injections of oil-water emulsion simultaneously into the hepatic vein and hepatic artery (33). In the present experiment, we have been unable to demonstrate any fibrin or fibrin-like thrombi in the liver. It is of interest to note that in the DCA-pretreated rats receiving renin, no thrombi were found in the liver, although they were present in the kidney and brain (9).

2. *Relationships of DCA-like factors and toxemia of pregnancy*

As just indicated, this relationship has received more arguments from analogy and speculation than it has from experiments. The evidences which might suggest participation of DCA-like factors of adrenal or even placental origin are wholly indirect (34–37), with the possible exception that toxemic woman excrete increased amounts of biologically active salt-retaining urinary corticoids (38).

In our study, the precipitation of a syndrome resembling eclampsia by administration of renin to DCA-pretreated rats (8) accorded with the clinical observation that administration of excess sodium and/or renal damage increase susceptibility to eclamptogenic toxemia. However, in the experimental disease we did not observe hepatic thrombi, central hepatic necrosis or instances of renal cortical necrosis such as occur in clinical cases of toxemia, so that the pattern was incomplete. In the DCA-treated rabbit, hepatic necrosis and renal cortical necrosis were elicited, although the analogy to human toxemia of pregnancy was in other respects

deficient. The changes in the electrophoretic pattern of serum protein elicited by DCA in rabbits were similar to those seen in clinical toxemia (39, 41).

It can be accepted that the occurrence of specific functional or anatomic changes in diseases of like etiology is a function in part of species-specific organ and tissue susceptibilities. In this respect the fact that, under appropriate conditions, DCA can elicit in rats and rabbits the array of clinical, functional and anatomic changes associated with eclamptogenic toxemia in human beings, is indirect evidence in favor of the participation of DCA-like factors.

This is exemplified in these experiments by the resistance of group III to the effects of DCA. Furthermore, the fact that DCA causes in rabbits changes closely resembling those of the spontaneous toxemia of pregnancy which occurs in this species, substantiates this point of view. Lastly, it seems likely that attempts to reproduce the whole pattern of human toxemia of pregnancy in subprimates are destined to end in such partial equivalences as are here described.

SUMMARY AND CONCLUSIONS

1. Administration of desoxycorticosterone (DCA) and salt to New Zealand white rabbits results in motor disturbances (paralysis and seizures) and death, and in pregnant does, death and retention of fetuses.

2. Serum sodium and chloride concentrations were not significantly altered; serum potassium was greatly decreased. The serum was lipemic, and hypercholesterolemic; ultracentrifugation of serum lipoproteins demonstrated high concentrations of components with fast flotation rates; electrophoresis of plasma proteins showed a decrease in albumin, resolution of α_1-globulin from albumin and an increase in concentration of β-globulin, fibrinogen and γ-globulin.

3. In order of severity and incidence, lesions were found in liver, kidney and heart. The hepatic lesions varied from dilatation of sinusoids and fatty vacuolization, to central and mid-zonal necrosis; the renal lesions varied from tubular dilatation with appearance of granular casts, to streaks and wedges of cortical necrosis; the cardiac lesions consisted of focal myocarditis.

4. The rabbit's response to DCA and salt differs significantly from that of the rat, dog and man.

5. DCA and salt do not reproduce more than a few of the characteristic changes of eclamptogenic toxemia in human beings; however, some are elicited; these, together with those found in DCA-treated rats given renin, nearly cover the array of abnormalities which occur in the clinical disease. This observation and the similarity between the lesions found in

spontaneous toxemia of pregnancy in rabbits and those elicited by DCA and salt, favor participation of DCA-like substances in the genesis of toxemias of pregnancy.

6. The importance of strain and species as determinants of susceptibility to experimental disease, is evidenced by these studies; it is suggested that perhaps no complete replica of human toxemia of pregnancy can be experimentally produced in subprimates.

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THE EXPERIMENTAL ADRENOGENITAL SYNDROME IN THE FROG*

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COMPARATIVE endocrinology has uncovered such diversities in the hormonal reactions of various vertebrate species, that inferences from animal experimentation in relation to normal or pathologic conditions in man, certainly should be drawn only with great caution. However, progress in this field already seems to indicate that large taxonomic groups adhere to one or another of a few endocrine types. Thus, striking similarities exist between hormonal responses in salamanders, birds, and marsupials (opossum), whereas ranid amphibians seem closer to the eutherian mammals. Whether such relationships have some evolutionary basis cannot as yet be said.

In the experiments here to be reported, significant parallels are seen in the reactions of frog larvae to estrogenic hormones on the one hand, and various aspects of the adrenogenital syndromes in man on the other.

MATERIAL AND METHODS

For these experiments groups of 50 to 200 tadpoles were raised in well aerated aquaria at a normal temperature, usually beginning at 18° and, toward metamorphosis, rising to 22 or 24° C. The tap water was dechlorinated by filtering through activated carbon, and aged two days in large tanks, under constant aeration. Several species of frogs were used. They give essentially identical reactions, though the threshold levels show some variations. The estrogenic substances estrone, estradiol, ethinylestradiol, and equilenin as well as stilbestrol, benzestrol, and fenocyclin (7-methyl-bisdehydrodoisinolic acid) were administered by dissolving them in the aquarium water. The solutions were renewed every second day. Both the experimental and the control tadpoles were given a varied diet of lettuce, red beets, seaweed and liver.

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EXPERIMENTAL

Condition of the adrenal and gonadal primordia at the start of the experiment

Treatments were started at the time of closure of the gill sacs, when the stores of yolk are nearly used up and the larvae begin to show an interest for food. The genital ridges are rising, receiving the large, yolk-laden primordial germ cells (Fig. 1). The left and right subcardinal veins have moved together and by fusing, have just given origin to the lowest segment of the vena cava. Accompanying and partly preceding the veins, the

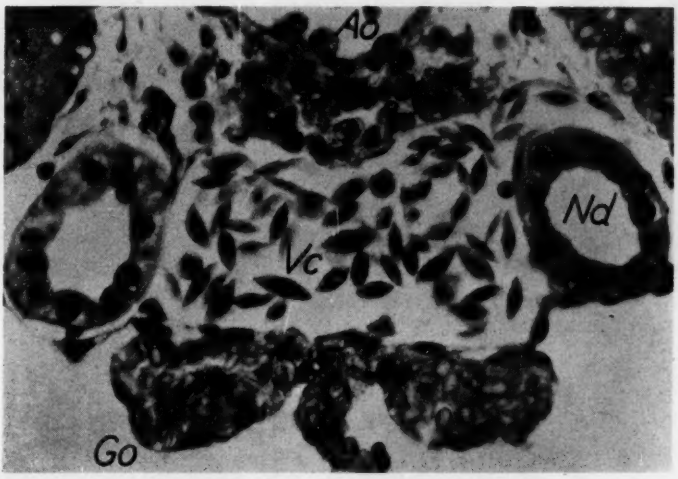


FIG. 1. *Rana temporaria* larva (Stage 24/25). Cross section, level of seventh somite. Unpaired mesonephric blastema cord between dorsal aorta (*Ao*) and caval vein (*Vc*). *Go* gonadal fold; *Nd* nephric duct; ×500.

mesonephric blastema cords also shift mesially. At this stage they have fused and form an unpaired plate which adheres to the dorsal wall of the caval vein (Fig. 1). The sixth and the seventh sympathetic ganglia are not yet organized, though scattered neuroblasts are found along the lateral and ventral wall of the descending aorta and around the root of the mesenteric artery.

The adrenals at the end of metamorphosis

a. *Controls* (Fig. 2). The mesonephric bodies of metamorphosing frogs are of a complex composition. As cross sections show (Figs. 2 and 3), the dominating elements are the loosely spaced urinary tubules, which open into the mesonephric ducts. Between them meander the venous sinuses, fed mainly by the supracardinal veins and draining into the interrenal

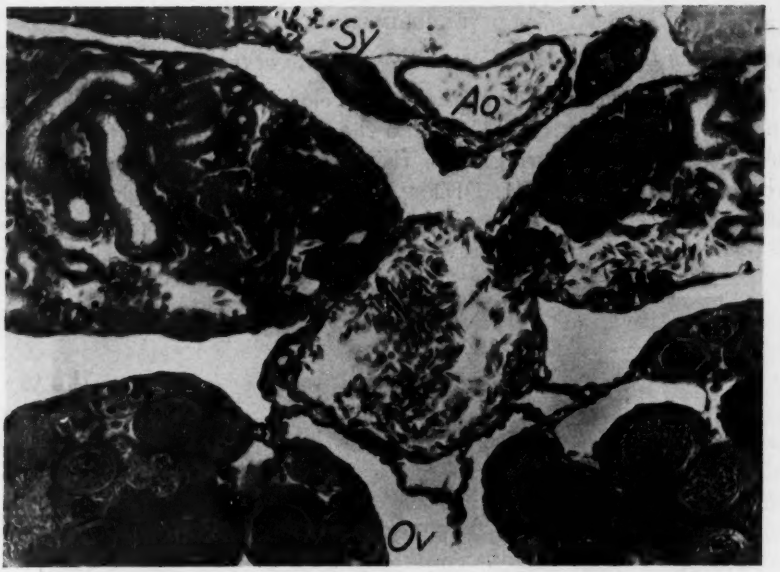


FIG. 2. *R. temp.* (Stage 31), metamorphosing female control. Arrows point at adrenals. *Ov* Ovary; *Sy* sympathetic ganglion VII; ×200.

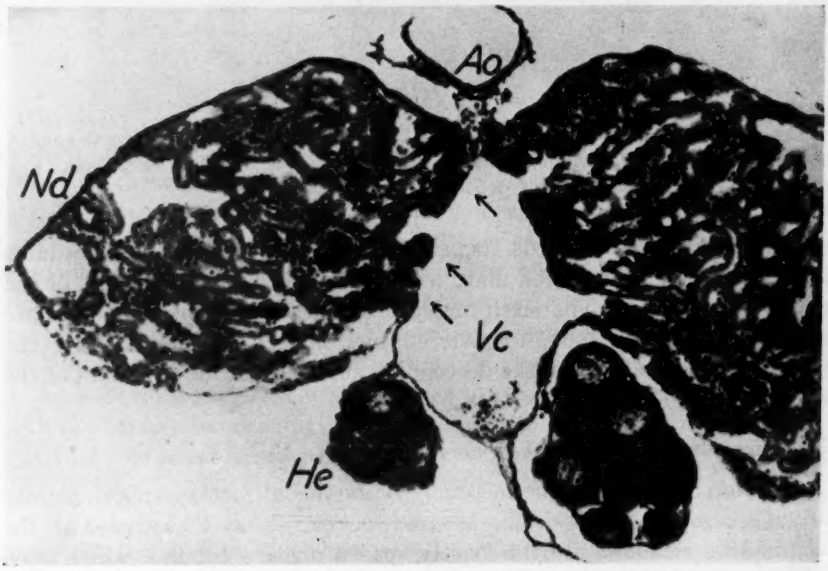
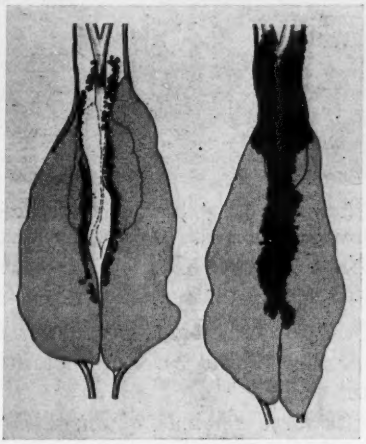


FIG. 3. *R. pipiens* (Stage 31); metamorphosing hermaphrodite (genetic male), raised in estradiol, 50 μg./L. Adrenal lobules enlarged (arrows). *He* hermaphrodite gland; ×110.

part of the caval vein. Along the ventral surface are distributed the nephrostomes which lead into the venous plexus. The dorsomedial edge of each kidney consists of a conglomeration of blastemic cells and rudimentary renal tubules and corpuscles. Beneath the ventral surface, but also widely scattered in the meshwork between tubules and sinuses, there are groups and layers of hemopoietic cells. The tissue bars between the numerous outlets of the blood sinuses into the caval vein are the seat of the islands of adrenal lobules (Fig. 2). These lobules are small and, in routine prepa-



a b

FIG. 4. Graphic reconstructions of the mesonephric bodies (grey, stippled areas), adrenals (black areas), sex glands (broken lines), and a part of the aortic system with root of mesenteric artery.

a. Control female *R. temp.* at metamorphosis stage.

b. Male *temporaria* raised in estradiol, 1 mg./L; ×18.

rations, not clearly separated from surrounding blastema cells, especially those belonging to the urogenital duct system. Since a special study of the development of the amphibian adrenal is in preparation, no detailed histologic description will be attempted here. Figure 4a shows the size and distribution of the lobules in a control frog near the end of the metamorphic period. The large caval vein, between the mesonephric bodies, now separates also the paired adrenals.

The sympathetic ganglia and nerves now are well differentiated. In addition to neurons they include also groups of chromaffine cells.

b. *Larvae treated with moderate amounts of estradiol* (Fig. 3). Metamorphosing frogs (*e. g., Rana pipiens*), which during the entire larval period had been kept in weak solutions of estradiol (20–50 μg./liter), show a

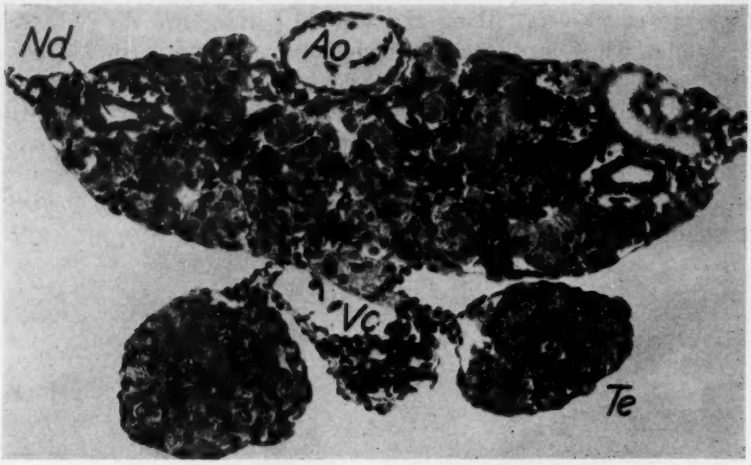


FIG. 5. Male *R. temp.* (estradiol, 1 mg./L) at metamorphosis stage. Cross section at the upper gonad level; nearly entire mesonephric blastema has differentiated into adrenal tissues. *Te* testis; ×200.

slight enlargement of the adrenals together with a ventral displacement of the caval vein (comp. Figs. 2 and 3). The separation of left and right adrenals is incomplete.

c. *Larvae treated with large amounts of estradiol* (Figs. 4b and 5–8). With concentrations of from 1.0 to 2.5 mg. of estradiol per liter of aquarium water, the larvae show an excessive hyperplasia of the adrenal glands. Precocious differentiation together with massive growth lead to the formation of an unpaired solid column, which only at its ends is slightly bifurcated (Fig. 4b). By volume, the increase is tenfold or more. The caval vein is displaced ventrally, into the mesentery between the sex glands. However, it is significant that the total size of adrenal plus meso-

FIG. 6. Cross section below the testes, otherwise as in Figure 5. Central adrenal mass (arrow). Caval vein displaced ventrally; ×100.

nephros is not appreciably changed. This means that the adrenal enlarges at the expense of the mesonephros. Some of the blastema cells of the primordial nephric cord, which normally would differentiate into urinary tubules, become adrenal lobules instead. In fact, the upper, narrowing parts of the normal kidneys may be entirely replaced by adrenal tissue; in Figure 5 the right side of the cross section shows exclusively adrenal tissue besides the nephric duct; the left side in addition, a nephrostome and two sections through a urinary tubule. The middle parts of the kidneys show numerous urinary tubules, though here again the adrenal appears enlarged at the expense of the mesonephros proper (Figs. 6 and 7). In larvae that were treated with the maximal dosage (2.5 mg. per liter) very often also the blastema of the urogenital cords (primordia of the efferent ductules and the gonadal medulla) differentiates into adrenal lobules and islands (Fig. 8).

All these observations prove that at the early larval stage (Fig. 1) the blastema cells still have the potentiality of differentiation into either urinary tubules, adrenal lobules, or efferent and medullary structures of the sex glands. At the same time they unequivocally disprove the common textbook view of the embryologic origin of the adrenal cortex from the peritoneal epithelium. Mesonephric tubules, urogenital cords (including gonadal medulla) and adrenal cortex derive from the intermediate or nephric blastema cords (1, 2). In frogs this entire intermediate mesoderm separates from the somitic cords and from the lateral plates (celomic or visceral mesoderm) already in early tailbud embryos.

A difference in the staining quality of ventral and dorsal parts of the adrenal body is noticed and may possibly represent a stratification similar to that of the mammalian cortex. The dorsal layer is histologically primitive like the mammalian glomerulosa (Fig. 6).

The mesodermal cortex is not the sole contributor to the adrenal enlargement. The sixth sympathetic ganglion is also involved, furnishing more neurocytes than normally. In fact, the histologic structure of the entire sixth ganglia is modified (Figs. 5 and 7).

The development of the normal and the hyperplastic adrenals during the larval period was made the object of careful study by Segal (3).

d. *Comparative studies with other estrogens* showed that estrone, ethinylestradiol, and equilenin produce the same kind of hyperplasia as estradiol, though with quantitative variations. On the other hand stilbestrol, benzestrol, and fenocyclin induce neither masculinization nor adrenal hyperplasia. This surprising contrast is not brought about, as one might suspect, by failure of the synthetic "mimetic" substances to remain in solution or through failure of the tadpoles to absorb them, for, at later stages, they stimulate the secondary female sex characters in the same manner as estradiol.

FIG. 7. Cross section through midgonad region, as in Figure 6. Adrenal mass at higher magnification; ×233.

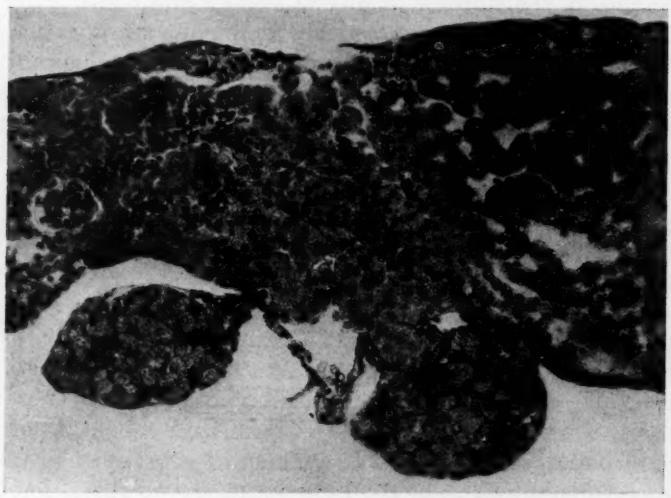


FIG. 8. Male *R. sylvatica* (estradiol, 2.5 mg./L). Cross section at midgonadal level. Adrenal differentiation penetrates hilum and medulla of left testis; ×120.

Sex differentiation

Genetic sex determination in frogs is relatively easily overridden by experimental agents. Whereas in mammalian fetuses only the development of secondary sex characters has been profoundly changed by the administration of sex hormones, in frogs the course of the differentiation of the sex glands themselves can be reversed. The appertaining literature was recently reviewed (2, 4). At the present we are exclusively concerned with the effects produced by estrogenic hormones. As Tables 1–3 show, weak solutions of estradiol give an increase in the frequency of hermaphrodites at the expense of the expected 50 per cent of males (Fig. 3). Somewhat higher concentrations result in complete feminization. The threshold values differ from species to species, but generally effects become noticeable at about 10 μg. per liter of aquarium water. Mortality as a rule is not above that of control groups. Consequently the increased number of hermaphrodites and females must consist of genetic males that were induced to produce hermaphrodite glands or ovaries instead of testes.

TABLE 1*. ESTRADIOL AND SEX DIFFERENTIATION IN *Rana sylvatica*

Estradiol (μg./L)	♀	⚥	♂	Effect
2,500	—	—	35	masculinization
1,250	5	101	17	partial masculinization
250	111	—	—	feminization
100	153	1	—	feminization
50	88	1	—	feminization
10	59	22	15	partial feminization
Controls	330	7	306	

* The data of Tables 1 and 2 are compiled from several experimental series; Table 3 gives the results from a single series.

TABLE 2. ESTRADIOL AND SEX DIFFERENTIATION IN *Rana pipiens*

Estradiol μg./L	♀	⚥	♂	Effect
1,250	3	4	33	masculinization
250	83	1	—	feminization
50	30	5	3	feminization
25	23	22	4	partial feminization
10	20	7	18	minimal feminization
Controls	125	4	113	

TABLE 3. ESTRADIOL AND SEX DIFFERENTIATION IN *Rana temporaria*

Estradiol μg./L	♀	⚥	♂	Effect
1,000	—	—	37	masculinization
25	58	6	4	feminization
Controls	39	—	21	

Surprisingly, the direction of sex transformation becomes reversed at the highest dosages. Again, taxonomic differences in the threshold values are noticed; but in all three investigated species, the concentration of 1 mg. of estradiol per liter of water produces either a partial or a complete masculinization (Tables 1–3). A difference between genetic males and females is not recognizable. At the dosage of 1.25 mg./L, *Rana sylvatica* produces a majority of hermaphrodites (Table 1); it appears that genetic females are just as easily masculinized as the genetic males; all seem to waver between feminization and masculinization without regard to hereditary sex constitution. *Rana temporaria* is by far the most easily masculinized of the three tested species (Table 3).

DISCUSSION

Adrenal enlargement in estrogen-treated frogs (*R. esculenta*) was observed already in 1938 by Padoa (5). It was not believed to be causally connected with the "paradoxical" masculinization effect. However, this negation was not compelling, being based on results of pioneer experiments with a chemically impure hormone preparation (Cristalovar), which actually contained only small amounts of some estrogen (6, 7). All chemically pure estrogens which at high dosages have resulted in masculinization of frog larvae, have also regularly produced adrenal hyperplasia, in Padoa's experiments as well as in ours. The enlargement is considerable, even at moderate dosage levels.

A satisfactory interpretation of the reversal of the direction of sex transformation at the highest dosages has not yet been found. In an earlier paper (4) it was suggested that the gonadal cortex might be adversely affected or even become completely destroyed by high concentrations of estrogens. Since the reduction of one component of an antagonistic pair must favor the other one, cortical deterioration would open the way for the development of the medullary component of the sex glands, and thus lead to masculinization. A second plausible explanation could be based on the concomitant adrenal hyperplasia. Assuming that eventually small amounts of androgenic hormones will be produced, testicular dif-

ferentiation would necessarily have to follow, even in the presence of the high concentration of the estrogens administered in this experiment; for it was shown (7) that a mixture of 250 μg. of estradiol[1] and 10 μg. of testosterone per liter is as completely masculinizing as the second fraction administered alone. It can be concluded that in such mixtures the androgens induce some primary reaction which eliminates the possibility of later estrogenic manifestations. Concentrations of methyltestosterone as low as 0.01 μg./L have been found to be masculinizing (2); also, in recent tests, adrenosterone had a much more pronounced effect upon gonad differentiation in frogs than one would expect on the basis of capon comb tests. Similarly favoring testicular differentiation are androsterone and the higher dosages of cortisone (8). The question of androgenic secretory activity of hyperplastic adrenals remains unsolved, whereas a direct masculinizing influence of high-dosage estrogens on the gonads seems established almost beyond doubt, through the following experimental series (a full report to be published separately):

Larvae deprived of hypophyses by removal of the placode at the earliest tailbud stage, differentiate ovaries or testes according to their genetic sex constitution. However, their adrenals are distinctly smaller than in controls and the lipid content of their cortical cells is reduced. If such hypophysectomized larvae are raised in high concentrations of estradiol, exactly as in the previously reported series with intact animals, then the adrenals remain unstimulated; but the genetic females nevertheless become masculinized. This proves, first, that the production of adrenal hyperplasia by estrogens is an indirect reaction, mediated by the hypophysis, and secondly, that the high doses of estradiol have a direct effect on sex differentiation, probably by adversely affecting the cortical inductor system of the gonads. Therefore, any possible androgenic activity of the hyperplastic adrenals in the first set of experiments could hardly be detected, because its manifestation would be concealed by the direct masculinizing effects of the administered hormone. New experimental setups will have to be arranged to obtain information concerning the physiology of the hyperplastic adrenals of estradiol-treated frogs.

In various respects the described reactions in the frog appear like experimental parallels to some special adrenal conditions in man. They may, therefore, become of value in the interpretation of abnormalities which in man are not usually accessible to experimental analysis.

The estrogen-induced hyperplasia in frogs is reminiscent of the excessive size which normally is attained by the *adrenals of human fetuses*. Since a drastic reduction starts immediately following birth—no matter whether

[1] In the original symposium report (7), the estradiol fraction erroneously was given as 2.5 mg./L.

the birth is premature, at normal term, or delayed—it is probable that directly or indirectly the prenatal enlargement is induced and maintained by pregnancy hormones. The smaller size of the adrenals in anencephalic fetuses indicates that fetal hypertrophy also depends on functions of the fetal hypophysis. The possible role of chorionic gonadotropin remains uncertain, but the frog experiment suggests that the estrogenic pregnancy hormones are importantly involved. No androgenicity connected with the regular fetal hypertrophy has so far been brought into evidence. The average normal enlargement therefore corresponds to the partial hypertrophy, which in frog larvae results from non-masculinizing dosages of estradiol.

Suggestive is also the high frequency of *neuroblastomas* which in fetuses and children appear associated in various ways with the adrenal medulla and often occur in combination with abnormalities of the cortex (9). Segal (3) reports that in the frog, estrogen treatments regularly cause an extensive proliferation of the 6th sympathetic ganglia.

The characteristic *adrenogenital syndromes in man* appear either in the form of cortical hyperplasia or of cortical tumor formation. Usually some male secondary sex characters develop or enlarge in the affected female or male individual. Kepler and Mason (10) found in most cases a considerable increase in urinary 17-ketosteroids. Androsterone excretion seems associated with hyperplasia, and dehydroisoandrosterone with tumors of the cortex. Here the androgenic function is well established. The similarity to the estrogen-induced syndrome in frogs is evident, even though in the latter the condition of the secondary sex characters has not yet been studied. It is generally agreed that a large majority of all cases of adrenal hyperplasia and tumors either are entirely congenital or develop from prenatally determined rudiments. Nevertheless, they usually are considered as quite apart from the regular fetal hyperplasia. In the light of our experiments it appears now that the differences may be only quantitative, *i.e.*, the development of the pathologic types may originate as exaggerations of the normal fetal hypertrophy. Accordingly, they should be causally linked to variations in the hormonal environment of the fetuses. This does not rule out the possible importance of genic mutations which, as in the Woolley and Little *ce* strain of mice (11), may in one way or another increase the responsiveness of cortical cells to special hormonal conditions. Hereditary predisposition in man is suggested by relatively frequent reports on repetitive occurrences of characteristic syndromes in some families. In frogs, factors of hereditary constitution were shown to determine different hormone-threshold-levels for the development of the various phases of the syndrome.

In the literature one meets a great deal of uncertainty regarding the

nature of *nodules or tumors of cuboid cells* which frequently occur, scattered between the gonads and the mesonephros, or lodged in the hilum and the medullary parts of the sex glands. There is a tendency to speak of interstitial-cell tumors (12) or Leydig-cell nodules (13) if found in combination with testes, and of virilizing adrenal rests (14) or sympathicotropic-cell tumors (15, 16) if found in ovaries. Testicular interstitial-cell tumors of similar type also have been produced experimentally by long continued administration of estrogens into male mice of the A strain (17). On the basis of our own experiments we do not doubt but that in all these cases one deals with extra-adrenal derivatives of the mesonephric blastema. Their particular location indicates that they are most closely related to that part of the blastema which ordinarily forms the urogenital cords. Whether considered as dislocated adrenal cortical cells carried along by the urogenital cords or as mutated gonado-medullary cells, it always can be assumed that unbalanced hormonal conditions started them on their course of anomalous development. Experimental cases of the type shown in Figure 8 lend support to this interpretation.

Since in the *adult adrenal* the most superficial glomerular layer retains a blastemic character and considerable regenerative capacity, it is conceivable that even in the absence of prenatally mutated cells, a long continued exposure to high estrogen concentrations may produce cortical hyperplasia and androgenicity at any age level. Obviously, this basic condition becomes established in the case of extended treatment of prostatic cancer with high dosages of estrogens. The usually observed decline in the initial repressive effect of the estrogen therapy may be a consequence of increased production of androgenic hormones in the adrenals. On the basis of the relationship just described between the hypophysis and adrenal hyperplasia, it is proposed to consider hypophysectomy as an alternative for adrenalectomy in the treatment of advanced prostatic cancer.

SUMMARY AND CONCLUSIONS

1. Evidence is presented for a common origin from the mesonephric blastema of urinary mesonephros, adrenal cortex, urogenital ducts, and gonadal medulla (including interstitial cells).

2. Of the same origin are also nodes, tumors, and cancers which consist of cells resembling interstitial cells of the gonads, or adrenal cortical cells, and which sometimes are found in the sex glands or along the urogenital connection.

3. Estrogenic steroid hormones administered to larval frogs cause the development of hyperplastic adrenals, partly at the expense of mesonephric and gonado-medullary differentiation.

4. Moderate concentrations of estrogens feminize genetically male frog

larvae. This apparently is the effect of direct inhibition of the gonadal medulla.

5. High dosages of estradiol produce a paradoxical masculinization of gonad development.

6. It is concluded that this reversal of the direction of sex transformation is due to direct repression of the ovarian cortex by very high concentrations of estradiol.

7. Hypophysectomy at the early tailbud stage retards the development of the adrenal cortex and of its lipid production.

8. Even very high dosages of estradiol do not induce adrenal hyperplasia in hypophysectomized larvae, but they have a masculinizing effect on gonad development.

9. Adrenal hyperplasia induced by administration of estrogen is an indirect reaction, mediated through the hypophysis.

10. The possible bearing of the results of these experiments on the interpretation of fetal hyperplasia of the adrenal in man, and on clinical cases of masculinization is discussed. The placental estrogens, with variations in their concentration and in fetal responsiveness, are considered to be a probable cause of congenital adrenal abnormalities in man.

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ADRENAL CORTICAL SECRETIONS IN RELATION TO THE REPRODUCTIVE SYSTEM OF RATS*

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I. INTRODUCTION

THE problem of the relationship of adrenal gland secretion to the state of the reproductive system is an old one that still requires adequate clarification. The able review of Parkes (1), to which the interested reader is referred for much literature that will not here receive attention, has brought into focus many of the fundamental problems. Among these, of particular interest at the moment, is the question whether adrenal secretions play an integral role in the activities of the reproductive system aside from maintaining the animal in such physiologic condition as will enable the normal function on the part of the gonads. Do adrenal glands secrete sex hormones?

The clinical literature since the beginning of the century affords some of the best evidence supporting the notion of adrenals secreting sex hormone-like substances, at least under some conditions. It has been clearly shown, especially in women, that hyperplasia or tumor formation in the adrenal cortex may be accompanied by recession of feminine characteristics (loss of menstruation) and the development of masculine qualities (coarsening of voice, hirsutism and clitorine hypertrophy), many of which changes may be obliterated by removal of the adrenal tumor. These changes are largely attributed to the secretion of androgens in the affected patient (for review of these problems *see* Grollman (2), Parkes (1) and Wilkins (3)).

Somewhat similar conditions are presented in the experimental animal (4–7). Gonadectomy immediately after birth in particular strains of mice has been followed within a few months by nodular hyperplasia in the adrenal cortex, leading to development of cortical carcinomas capable of metastasizing. Accompanying such tumor formation the reproductive

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accessories of the castrated mice were found to approach the size and histologic condition of those in normal animals, giving evidence of the presence in the castrated organism of sex hormone-like substances.

Experimental studies in this laboratory have long been concerned with problems of hormone secretion and their effects during embryonic development and early postnatal stages (8–11, 12–15). Certain aspects of the general problem have been aided through the transplantation of gonads or accessory reproductive organs under a variety of conditions (16, 17). Transplantation of embryonic rat testes to the walls of seminal vesicles of adult castrated hosts, begun in 1948, demonstrated a very sensitive test for androgen secretion and this procedure was likewise utilized to determine whether transplanted adrenal glands secreted androgens. Until now, our own results with this technic have not been published, but the characteristic findings have been published by Jost. Jost (18) and Jost and Colonge (19) demonstrated that rat embryo testis transplanted to the seminal vesicles of castrated hosts resulted in stimulation of seminal vesicle epithelium located immediately next to the testis graft; that this androgenic effect was sharply localized and did not extend to more distally located acini; and that hyperactivity of the castrate host pituitary gland was required for the precocious androgen secretion by the testis graft. Our observations have confirmed Jost's important contribution and have further shown that adrenal gland transplants do not produce detectable androgenic responses. Still other unpublished studies have involved inquiry into the effects upon reproductive tissues of hormones associated with adrenal functions—cortisone and adrenocorticotropic hormones.

It has appeared of interest, therefore, to attempt to bring together results so far obtained from these several lines of experimental treatment that have a bearing on the problems of the relation of the adrenal to the reproductive system. Further studies should be directed to effects of other substances extracted from the adrenal, but in the following pages some observations will be presented on hormone secretion by the embryonic testis and by adrenal transplants, as well as on the effects of cortisone, ACTH or adrenalectomy on the reproductive system. The results obtained have come from a study of more than 300 rats.

II. TRANSPLANTATION OF TISSUES TO SEMINAL VESICLES OF CASTRATED RATS

Testes, as well as other tissues from rat embryos, were transplanted to the seminal vesicles of hosts castrated for sufficient periods (twenty-three to one hundred and ten days) to insure an inactive target organ. The donor tissues were dissected from embryos on the 15th to 16th day after witnessed matings. The transplants were inserted into a pocket tunneled

underneath the serous covering layer of the castrate seminal vesicle and were left as grafts for periods of six to fourteen days. At autopsy a piece of seminal vesicle wall including the graft site, was removed, fixed in Bouin's solution and sectioned serially. Thus the graft site, including the graft when persistence occurred, was available along with the detector organ to note possible secretion of androgenic substances.

Such other organs as the adrenal, ovary or liver were transplanted in a similar manner and in all cases to the walls of seminal vesicles of previously castrated hosts. Except for the adrenal, the majority of transplants were obtained from embryos of known age; but for most of the adrenal transplants, and some liver transplants, donor tissues were from young postnatal animals. Usually one graft was made in the wall of each seminal vesicle of the host, the two transplants being of the same type of tissue in some cases; but in others, testis was transferred to one seminal vesicle and ovary, and liver or adrenal to the opposite vesicle. The data for all transplants are summarized in Table 1, including some indication of the effects observed.

A. *Embryonic testis transplantation*

From Table 1 it is apparent that of 22 transplantations of embryonic testes, 19 yielded grafts containing recognizable testis tissue at the time of recovery, six to fourteen days after the original transfer. In the poorer grafts usually only a few seminiferous tubules were present, sometimes dispersed by intervening connective tissue and occasionally revealing invasion of round cells and macrophages; the good-to-excellent grafts consisted of well preserved, compact masses of seminiferous tubules, in many of which mitoses indicated the earlier stages of progressive spermatogenesis. Interstitial-cell activity was notable in some, but not in all of the better grafts.

The effect of the transplanted testis on the seminal vesicle was judged by the state of the epithelium located in close proximity to the testis graft, or separated from it slightly by intervening connective tissue. In the case of recovered testis grafts the table shows that 18 of the 19 grafts containing seminiferous tubules exercised a detectable androgenic effect upon the target organ. In some of the better seminal vesicle responses the acinar epithelium showed typical secretion granules, when stained with Ehrlich's hematoxylin (20). In the serially sectioned graft and target organ the epithelium of the seminal vesicle near the graft showed a normal secretory state, whereas the epithelium of acini further removed from the graft was typically castrate in type. Frequently within the same histologic section, acini typical for normal males were seen in close proximity to the graft, whereas others further removed were of the castrate type.

Turning for specific detail to a few examples, Figures 1 and 2 each show the character of a testis graft ten days after transplantation from an embryo 15 days and 16 hours post coitum onto the seminal vesicle of an adult host castrated thirty-eight days previously. In each of these 2 different cases the acinus contiguous to the testis graft possessed seminal vesicle epithelium in a secretory state, entirely similar to that found in the normal adult male. Acini within the same histologic section, but further removed from the testis graft, contained an epithelium either partially or wholly castrate in type. In these 2 cases the host at autopsy had been castrated for forty-eight days, and

TABLE 1. RESULTS FROM TISSUE TRANSPLANTATION IN RATS

Series No.	Embryol. age of donor (da.+hrs.)	No. of days graft persisted	Length of time since host castrated (days)	Graft	Sem. ves. response
A. *Testis transplantation*					
1603 A	15+16	7	23	*	+
1603 B	15+16	7	23	**	+
1604 A	15+16	10	48	**	++
1604 B	15+16	10	48	**	+++
1605 A	15+16	10	48	**	+++
1605 B	15+16	10	48	**	+++
1606 A	15+16	10	48	**	+++
1606 B	15+16	10	48	**	+++
1608 A	15+16	13	60	0	0
1608 B	15+16	13	60	0	0
1610 A	15+16	13	31	**	+++
1610 B				epidid.	+
1610 C	15+16	13	31	0	0
1615 A	14+12	14	85	*	+
1615 B	14+12	14	85	*	+
1616 A	14+12	14	85	**	+++
1616 B	14+12	14	85	**	+++
1617 A	15+15	6	90	*	+
1618 A	15+15	6	90	*	0
1624 A	15+12	6	100	**	++
1625 A	15+12	6	100	*	++
1626 A	15+12	6	100	**	+++
1627 A	15+12	6	100	*	++
B. *Adrenal transplantation*					
1619 A ♂	15+15	16	90	**	0
1619 B ♂	15+15	16	90	**	0
1620 A ♂	7 days pn§	14	100	0	0
1620 B ♂	7 days pn	14	100	0	0
1621 A ♂	7 days pn	14	100	0	0
1621 B ♂	7 days pn	14	100	0	0
1622 A ♂	7 days pn	14	100	0	0
1622 B ♂	7 days pn	14	100	0	0
1623 A ♀	7 days pn	14	100	*	0

* Graft persisted as recognizable tissue, poor-to-fair.
** Graft showed excellent tissue.
\+ Weak seminal vesicle reaction.
++ Moderate-to-good seminal vesicle response.
+++ Excellent response to normal secretory condition.
§ pn = post natal.

TABLE 1—(*continued*)

Series No.	Embryol. age of donor (da.+hrs.)	No. of days graft persisted	Length of time since host castrated (days)	Graft	Sem. ves. response
1623 B ♀	7 days pn	14	100	*	0
1628 A ♀	18 days pn	12	32	*	0
1628 B ♀	18 days pn	14	32	0	0
1629 A ♀	18 days pn	14	32	0	0
1629 B ♀	18 days pn	14	32	*	0
1632 A ♂	7 days pn	14	110	**	0
1632 B ♂	7 days pn	14	110	*	0
1633 A ♂	7 days pn	14	35	*	0
1633 B ♂	7 days pn	14	35	**	0
1634 A¶ ♂	21 days pn	14	35	*	0
1634 B¶ ♂	21 days pn	14	35	0	0
1769# ♂	34 days pn	14	44	*	0
1770# ♂	34 days pn	14	44	0	0
C. *Liver transplantation*					
1617 B	15+15	6	90	*	0
1618 B	15+15	6	90	*	0
1624 B	15+12	6	100	*	0
1625 B	15+12	6	100	*	0
1626 B	15+12	6	100	*	0
1627 B	15+12	6	100	*	0
1630 A	18 days pn	14	32	0	0
1630 B	18 days pn	14	32	0	0
1631 A	18 days pn	14	32	0	0
1631 B	18 days pn	14	32	0	0
D. *Ovary transplantation*					
1607 A	15+16	10	48	*	0
1607 B	15+16	10	48	0	0
1609 A	15+16	13	31	0	0
1609 B	15+16	13	31	0	0
1611 A	15+15	13	42	0	0
1611 B	15+15	13	42	*	0
1612 A	15+15	13	42	*	0
1612 B	15+15	13	42	0	0

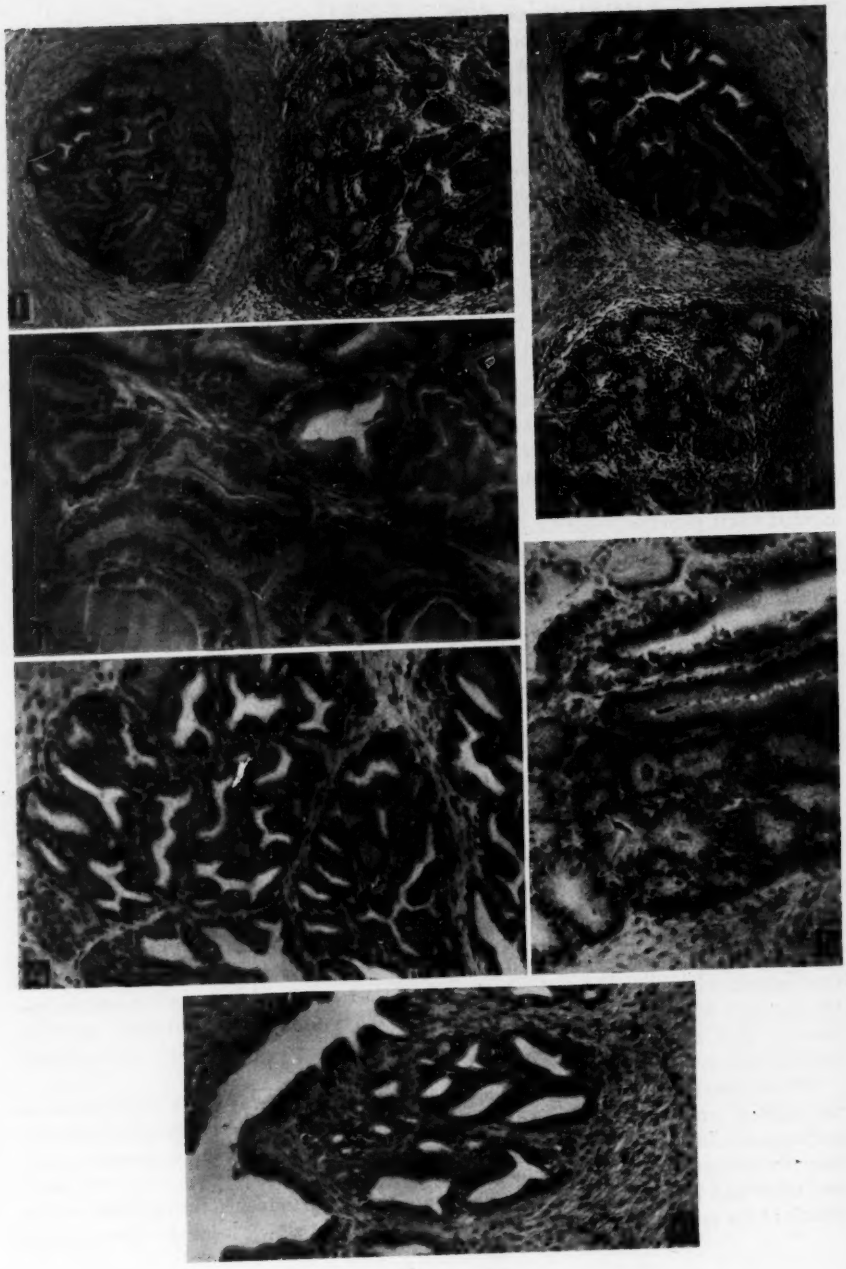
¶ Half adrenal.
# Autotransplant.

the chronologic age of the testis, persisting as a graft for ten days, was 25 days+16 hours post coitum, or equivalent to approximately 3.5 days after birth. The testis grafts had secreted androgens which diffused through the surrounding tissue and stimulated the seminal vesicle epithelium to a normal secretory condition. No attempt was made to determine the distance through which hormone diffusion could occur and exert an effect, but the presence of more distally located acini with a castrate type of eptihelium suggested but a small area of diffusion.

Figures 3 and 4 present, under slightly higher magnification, the character of the epithelium from the two opposite seminal vesicles in another host, castrated for a total of thirty-one days. Thirteen days prior to autopsy each seminal vesicle was the site for transplantation of a testis from an embryo 15 days+16 hours post coitum. Sections through the seminal vesicle walls in the area of the transplant revealed a good testis graft near the acinus shown in Figure 3 but the graft in the opposite seminal vesicle had failed to persist; debris and macrophages marked the area of graft degeneration. The acinus (Fig. 3) contiguous to the testis graft revealed well stimulated seminal vesicle epithelium with the nucleus occupying approximately one-third the height of the cell, whereas the acinus (Fig. 4) from the opposite seminal vesicle next to the degenerate graft site, contained an epithelium of a typical castrate type in which the total height of the cell was occupied by the nucleus. Thus, a testis graft (chronologic age of testis approximately 6 days after birth) actively secreted androgen to the point of inducing a normal adult secretory condition in seminal vesicle epithelium near the graft, without having a detectable effect upon cells in the seminal vesicle of the opposite side in which the graft failed to persist; the androgens produced thus exerted a local effect rather than an effect due to distribution of the hormone through the blood stream. Other acini in the same seminal vesicle, but more distally located than that shown in Figure 3, were in a castrate condition. Thus the limited diffusion of androgens within the walls of the same seminal vesicle emphasizes again the distinctly localized effect of hormone liberated from the graft.

Figures 5 and 6, while illustrating again the phenomenon of local diffusion, at the same time emphasize the ability of the embryonic testis to secrete androgens precociously under the special conditions. In Figure 5 seminal vesicle epithelium in an adult host, castrated for a total of one hundred days, is seen to be in a secretory state. This acinus was located next to a testis graft that had been present for but six days subsequent to transplantation of the testis from an embryo 15.5 days post coitum; the chronologic age for the testis was therefore 21.5 days post coitum, which is the approximate period of birth. It must be emphasized that such precocious androgen stimulation was shown by Jost and Colonge (19) to depend upon the pituitary of the castrated host. The acinus shown in Figure 6 was located next to the graft site in a seminal vesicle from an adult host castrated for ninety days, that had received the transplant of a testis from an embryo 15 days+15 hours post coitum. Recovery six days after transplantation, however, revealed that the testis graft had degenerated; the castrate character of the acinar epithelium is evident.

These observations emphasize, therefore, that embryonic rat testis transplanted to the walls of seminal vesicles of adult castrated hosts is capable of secreting sufficient androgenic substances to stimulate the seminal vesicle epithelium to a secretory state; they also demonstrate that the androgens liberated from the graft exert a purely localized effect and that blood titers of hormone are too low to influence the opposite seminal vesicle of the same host, or even acini of the same seminal vesicle located a short distance



from the graft. These facts suggest an appropriate procedure for determining the capacity of the adrenal gland to secrete androgens.

B. *Adrenal gland transplantation*

Adrenal glands were transplanted into subperitoneal pockets on seminal vesicles of castrated rat hosts in a manner entirely similar to that of testis transplants. Table 1B shows that in 2 cases donor tissues were obtained from embryos on the 16th day, whereas donor adrenals for 20 transplantations were provided by postnatal males and females 7 days to 34 days after birth. Among the 22 sectioned adrenal transplant sites, recognizable adrenal tissue was present in but 12 cases. In approximately one half of the transplant sites, therefore, live adrenal tissue was absent and the graft site was infiltrated rather heavily with round cells, macrophages or fibrous tissue.

The 12 adrenal grafts recovered in the walls of the seminal vesicles at autopsy fourteen to sixteen days after transplantation were rated as poor-to-fair in 8, and good-to-excellent in 4 cases (Table 1B). These ratings depended upon finding that in the majority of the grafts only small amounts of living tissue had persisted, and that this tissue usually was infiltrated with round cells and macrophages, or appeared to be undergoing gradual replacement. In some, the cortex and medulla were recognizable (Fig. 7) but in others only cortex appeared to persist. In some cases cortical zonation was clearly indicated. In the good-to-excellent grafts larger amounts of adrenal tissue were present, consisting of cells well maintained in one large, or two or more separate, masses. Cortex alone was recognized in some grafts, with or without typical zonation, whereas in others both cortex and medulla were evident.

Figure 7 shows a photomicrograph of a section of an adrenal graft persisting for sixteen days after original transplantation from a male embryo on the 16th day of development (Ser. 1619 B). The characteristic cortex, having a fairly distinct capsule and a good degree of zonation, can be seen to surround a central medullary portion. The zona glomerulosa is not entirely typical but the fasciculata is definite and the cells reveal a definite vacuolated condition.

The last column in Table 1B indicates that the epithelial lining of the acini of the seminal vesicles was castrate in type in all cases of persisting adrenal tissue; no differ-

FIG. 1. A section through the wall of seminal vesicle of rat castrated 48 days, showing portion of testis graft contiguous to seminal vesicle acinus (left). Donor testis from embryo 15 days+16 hours post coitum; graft persistence 10 days; acinar epthelium in high secretory state (Ser. 1604 B) ×100.

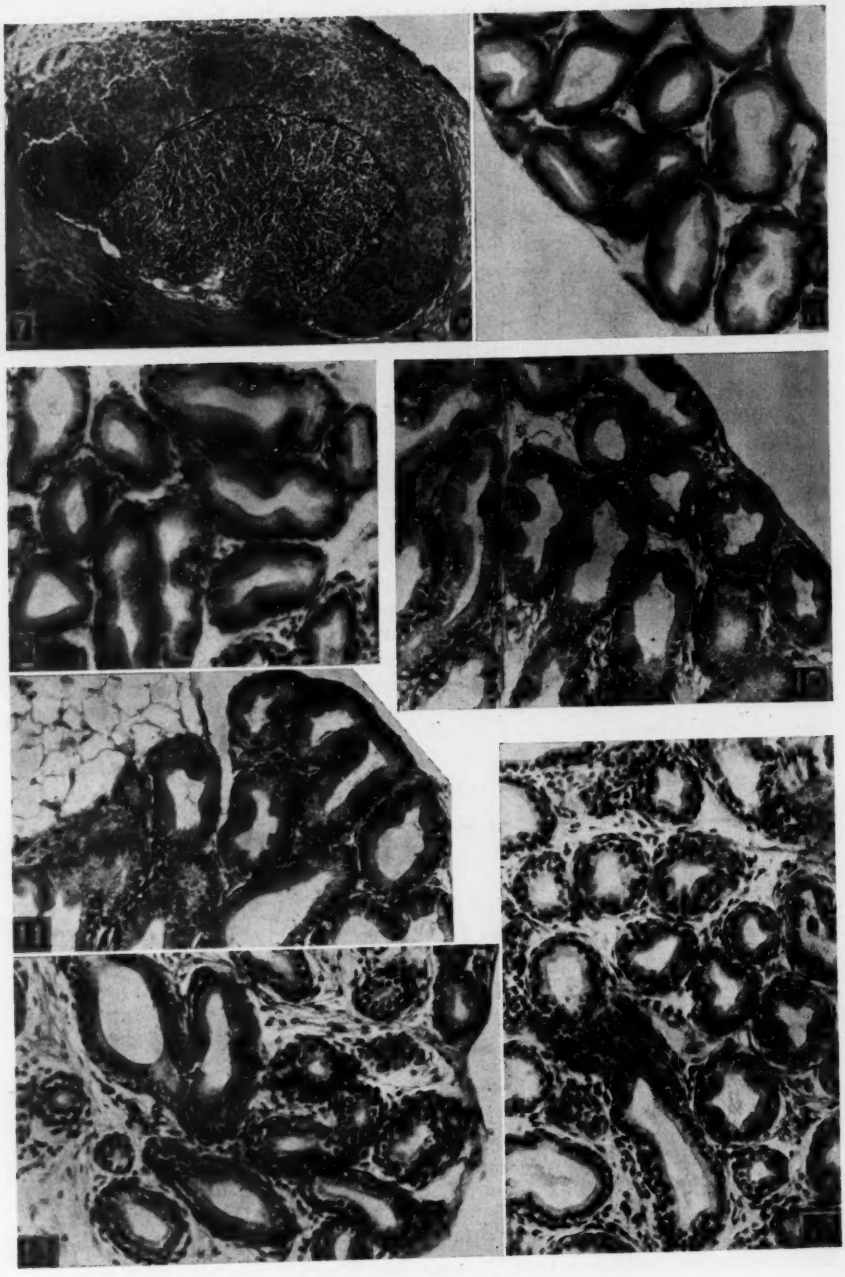
FIG. 2. History similar to that in Figure 1. Small part of testis graft below (Ser. 1605 B) ×100.

FIG. 3. Section through seminal vesicle acinus contiguous to testis graft persisting 13 days. Donor testis from embryo 15 days+16 hours post coitum; host, adult male castrated 31 days; acinar epithelium in high secretory state (Ser. 1610 A) ×230.

FIG. 4. Acinar epithelium from opposite seminal vesicle of same host as in Figure 3, in which testis transplant failed to persist. Epithelium of castrate type. Compare with with Figure 3 (Ser. 1610 C) × 230.

FIG. 5. Acinar epithelium contiguous to testis graft. Donor testis from embryo 15½ days post coitum; adult host castrated 100 days: graft persistence 6 days (chronologic age of testis equivalent to that at birth). Epithelium in secretory stage (Ser. 1626 A) ×230.

FIG. 6. History similar to that in Figure 5, except testis transplant failed to persist. Acinar epithelium castrate in type. Compare with Figure 5 (Ser. 1618 A) × 230.



ence was noted in this target organ, whether a living graft was present or all adrenal tissue had been removed. There was thus no indication in this series of adrenal grafts that androgen had been released. Since the mass of adrenal graft tissue present was equal to or exceeded some of the testis grafts that had exerted a detectable effect upon the seminal vesicle epithelium, it is suggested that the adrenal grafts were not liberating androgenic substances locally in sufficient amounts to stimulate demonstrably the seminal vesicle epithelium.

C. *Liver transplantation*

As a further control for testis transplantation, liver tissue was used in 10 cases; 6 transplants were made from embryos on the 16th day and 4 from an 18-day postpartum donor. Table 1C reveals that from the 10 recovered graft sites, liver cells were recognizable in 6 cases. The grafts, however, are indicated as poor-to-moderate, since in no case were good, compact, healthy-looking masses of liver present. When present at all, the cells were usually arranged in a cord-like mass, heavily infiltrated with round cells and macrophages interspersed among considerable cellular debris and fibrous connective tissue.

On the whole, therefore, liver persisted poorly in the graft; and in all cases in which it was present, the graft had persisted for only six days after transplantation. The results suggest that all liver tissue would have been displaced within a short period of time. In all cases of liver transplantation the seminal vesicle epithelium was found to be castrate in type.

D. *Ovary transplantation*

Eight ovaries from embryo rats were transplanted onto the walls of seminal vesicles of castrated hosts and the graft site recovered ten and thirteen days later. All donor ovaries transplanted were obtained from embryos during the 16th day.

Persistence of transplanted embryonic ovaries was very poor. Three cases are indicated in Table 1D as poor-to-fair grafts. In no case was ovarian cortex recognized, and no follicles were present. The ovarian remnants consisted of a few small epithelial-like tubules and small amounts of ovarian connective tissue. The tubules represented either

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FIG. 7. Adrenal graft in wall of seminal vesicle of adult castrated male rat, showing cortex and medulla. Donor embryo 15 days+15 hours post coitum; host, castrated 90 days; graft persistence 16 days (Ser. 1619 B) × 105.

FIG. 8. Section through ventral prostate gland of male castrated on day 14. Autopsy, day 24 (Ser. 1772) × 230.

FIG. 9. Section through ventral prostate gland of male castrated on day 14; 9 daily treatments with 2.5 mg. of ACTH (104 per cent Armour Standard). Autopsy, day 24 (Ser. 1774) × 230.

FIG. 10. Section through ventral prostate gland of male rat castrated on day 20. Autopsy, day 29 (Ser. 1801) ×230.

FIG. 11. Section through ventral prostate gland of male rat, castrated and adrenalectomized on day 20. Autopsy, day 29 (Ser. 1802) ×230.

FIG. 12. Section through ventral prostate gland of male rat castrated on day 21. Autopsy, day 34 (Ser. 1764) × 230.

FIG. 13. Section through ventral prostate gland of male rat castrated and adrenalectomized on day 21. Autopsy, day 34 (Ser. 1766). ×230.

persistent and converted medullary tubules or else remnants of the epoophoron. In any event, the grafts were unimpressive and served to recall the relatively poor persistence of ovarian cortex from our earlier experiences with transplantation of ovaries. All grafts, and graft sites, were accompanied by seminal vesicle acini in which the lining epithelium was of castrate type.

Thus in transplants of ovarian tissue, admittedly too few in number, no evidence was seen to suggest androgen secretion.

In this series of tissues transplanted into the walls of castrate seminal vesicles, embryonic testis tissue gave the best persistence, followed in turn by adrenal, liver and embryonic ovary. Only in the case of testis grafts was a sufficient amount of androgen secreted and liberated locally to register an effect upon the nearby seminal vesicle acini. The action of the testicular secreted androgen was sharply localized and did not extend to all acini in the same microscopic section; in no case was an effect evident on the opposite seminal vesicle unless it, too, contained a persistent testis graft. No evidence was found to suggest androgen secretion by adrenal grafts which consisted of either cortex or cortex along with medullary tissue, nor was such evidence found in connection with transplanted ovaries. Liver tissue likewise provided no evidence of having stimulated the seminal vesicle epithelium of the host.

III. TREATMENT WITH CORTISONE

One approach to an investigation of the possible role exercised by adrenal secretions on the reproductive system would be to study the effect of all substances produced or extracted from the adrenals, singly or in various combinations; it is to be remembered that of all products isolated from adrenal tissues, no one of them alone represents a satisfactory adrenal replacement. A second approach would be through stimulation of the intact adrenal, possibly demonstrating unusual activity on the part of the reproductive system from hypercorticalism. Utilization of adrenocorticotropin is one means of stimulating adrenal activity. A third possible approach—that of adrenalectomy with maintenance of the animal in a fair state of physiologic well being—would reveal the effects upon the reproductive system of a lack of such secretions.

The recent availability of some adrenal products permits an approach to the problem of the relations of adrenal secretions to the general state and function of the reproductive tissues. In this section there is presented a somewhat limited study of the possible effects of cortisone on the reproductive system.

The qualitative nature of the experiments should be emphasized by noting that only two constants were present, a) the species of animal maintained under similar conditions, and b) the substance employed for

subcutaneous injection—cortisone. Variables in the animals employed included age (autopsy ages ranged from 20 days to adulthood), sex, and the presence of gonads; variation in treatment included dosage (0.5 mg. to 5.0 mg. daily) and length of treatment (seven to fourteen days).

Twenty-two litters, or groups, containing 127 animals were utilized for studies on cortisone effects, including 68 treated animals and 53 appropriate controls. A general summary of the treatments and of the results noted are arranged in Table 2. Each horizontal line refers to a group and the results are expressed in terms of percentage change of weights of fresh tissues from treated animals in comparison with their appropriate controls. In order to gain some adjustment for loss in general body weight of treated animals, actual fresh weights of different organs were converted into milligrams of organ weight per 100 grams of body weight. Averages

TABLE 2. EFFECT OF DAILY SUBCUTANEOUS INJECTIONS OF CORTISONE IN RATS

No. of animals treated	Treatment		Age at autopsy	Av. body wt. of controls (Gm.)	Percentage change in treated animals						
	No. of days	Daily dose (mg.)			Body wt.	Adrenals	Testes	Ventral prostate	Seminal vesicles	Ovary	Uterus
				A. *Intact males*							
1	12	1.25	24 da.	29	−48	−62	+ 7	−78	−37		
2	7	2.00	25 da.	40	−47	−50	− 6	−50	+23		
2	12	1.25	29 da.	51	−49	−41	−10	−44	+27		
6	8	5.00	34 da.	73	−40	−34	+30	−20	−33		
3	11	2.50	36 da.	84	−51	−33	−21	−45	−35		
5	9	5.00	65 da.	145	−20	−34	+15	+42	+10		
4	10	5.00	adult	302	− 1	−40	+26	+19	−14		
				B. *Castrated males*							
2	9	0.50	20 da.	40	−46	−58		−66	− 2		
3	10	5.00	adult	317	−16	−32		−34	+17		
6	10	5.00	adult	339	−19	−34		+15	+31		
				C. *Intact females*							
2	9	0.50	20 da.	36	−33	−66				+ 7	+25
3	12	1.25	24 da.	45	−57	−23				+ 96	+29
4	7	2.00	25 da.	37	−44	−18				+ 13	−20
2	8	5.00	34 da.	71	−47	−20				+142	+ 3
2	11	2.50	36 da.	67	−47	−36				+ 58	− 7
2	7	3.00	55 da.	110	+13	−38				+ 17	−23
2	14	3.00	62 da.	141	−38	−54				+ 45	−39
2	7	1.00	4 mos.	152	+ 3	−12				0	+10
2	14	1.00	4 mos.	169	− 9	+ 2				+ 30	−35
4	10	5.00	adult	223	−17	−39				+ 3	−12
				D. *Spayed females*							
3	10	5.00	adult	241	−17	−55					− 1
6	10	5.00	adult	234	−16	−44					− 8

of these different converted weights were therefore used for comparison between treated animals and controls, and the results have been expressed as deviations of treated from normal control tissue weights. Thus, referring to the last group in Table 2 A, the data show that 4 adult males were given 10 daily subcutaneous injections of 5.0 mg. of cortisone and were killed as adults. The average body weight for the 4 controls was 302 grams; body weights of treated animals were 1 per cent below those of the controls, converted adrenal weights showed a 40 per cent reduction from control adrenals, testes an increase of 26 per cent, ventral prostates an increase of 19 per cent, and seminal vesicles a decrease of 14 per cent.

A. *Body weight:* A survey of the effect of cortisone on body weight during the rather short periods of treatment indicates a depressing effect on treated members for practically all groups; a single litter among the 22 groups (55-day, normal females, 3 mg. per day) revealed that treated females were 10 per cent heavier than the controls, whereas 18 groups showed decreases in the weight of treated animals of 10 per cent or greater; changes of less than 10 per cent are probably without significance. The effect of cortisone in inhibiting growth in the rat has been pointed out by several workers since it was first noted by Wells and Kendall in 1940 (for references, *see* Ingle (21)); it also inhibits the growth of chick embryos (22).

B. *Adrenal weight:* Cortisone had the consistent effect of reducing the relative (and actual) fresh weight of the adrenals. In all but 1 of the 22 groups shown in the table, reductions in adrenal weights were of the order of 10 to 66 per cent; a single group gave an adrenal weight 2 per cent greater than in the control. Reduction in adrenal weight is in keeping with many reports previously published.

C. *Gonad weight:* Changes in the fresh weight of testes from animals treated with cortisone failed to show any specific trend. Among the 7 groups of intact males, increases in testes weights of 10 per cent or more above those of controls occurred in 3 treated groups, decreases of 10 per cent or more were shown in 2 groups and changes of less than 10 per cent were found in 2 groups. Histologic study did not reveal any apparent modification. In the case of the greatest testicular increase (30 per cent, autopsy day 34, eight daily injections of 5.0 mg.) the organs histologically were normal in both treated animals and controls. Secondary spermatocyte divisions and spermatids were present, although "sperm head" stages were not present (elongate, chromatic nuclei of metamorphosing spermatids (23)) but would be expected within the course of from two to three days; they were just appearing in the group sacrificed on day 36. Interstitial-cell modification was not noted.

Ovarian weight increase in treated females was a distinct trend in the 10 treated litters or groups tested. Seven of the 10 groups gave converted ovarian weights of from 13 to 142 per cent greater than those of the controls, 2 groups gave less than 10 per cent increase, and in 1 group the relative ovarian weight was unaffected. The groups included autopsy ages ranging from 21 days to adulthood.

Histologic study suggests that weight increases in the ovaries of treated females are due largely, if not entirely, to a greater number of follicles with antra, and a greater size of the antrum cavity. In ovaries of animals sacrificed on days 24, 34 or 36, when ovarian weights showed the greatest increases, corpora lutea were not present in treated or control ovaries. Many atretic follicles were visible in ovaries from each group without any characteristic histologic difference being apparent. Increased size and greater numbers

of antrum-bearing follicles, therefore, appear to account for ovarian weight increases following treatment with cortisone.

D. *Hormone secretion:* Testis hormone secretion was in no way stimulated by treatment of intact males with cortisone. In fact, the converted fresh weights of ventral prostates and seminal vesicles tended to be lower in treated males than in controls, suggesting that if any real effect was to be noted it was one of inhibition of androgen secretion. Whereas converted prostate weights showed 20 per cent to 78 per cent decreases in treated males in 5 groups and increases of 19 per cent to 42 per cent in 2 groups, these changes were frequently opposite to those shown by seminal vesicle weights. Thus sacrifice on days 25 and 29 produced decided reductions in prostate weight but an increase of almost the same proportions in seminal vesicle weight. Furthermore, in the group which came to autopsy on day 34, after eight daily injections of 5.0 mg. of cortisone, the greatest weight increase was accompanied by reductions in both prostate and seminal vesicle weights.

Histologic study failed to reveal characteristic differences in the ventral prostate and seminal vesicles of the treated males, when compared with control organs. The acinar epithelium of the ventral prostates in all cases contained a high secretory epithelium with pronounced "light areas," but up to day 36 the seminal vesicle epithelium was relatively inactive, not yet having been exposed to a sufficiently high hormone titer to stimulate secretory changes. In the 65-day group (nine daily treatments with 5.0 mg.) the epithelium of the seminal vesicles was characteristically high columnar, and differed in no material way in the treated and control members. No histologic evidence was noted for either a stimulation or depression of hormone secretion by treatment of intact males with cortisone.

Ovarian secretions were not perceptibly modified by cortisone. Weights of uteri from treated females deviated but little from controls and displayed decreases about as frequently as increases. Modifications in histologic character were not evident.

E. *Castrated males:* Cortisone was administered to 3 groups of castrated males containing 11 treated animals and 11 controls, without obtaining suggestions of definite effects. The prepuberal group had prostates in treated males averaging considerably less in weight than in controls, without change in weight of seminal vesicles. In 2 adult groups castrated for longer than fifty days prior to ten daily treatments with 5.0 mg. of cortisone (daily equivalent of approximately 16 mg./Kg.), one group of 3 treated males showed a reduction in prostate weight from that of the controls but an increase in weight of the seminal vesicles; in the second group of 6 treated males both the ventral prostates and seminal vesicles revealed an average increase over untreated controls.

Histologic study of the accessory reproductive structures failed to reveal either a stimulating or a depressing effect from treatment, in either the young or the adult group. Ventral prostates in the group which came to autopsy on day 20 showed high acinar epithelium in both controls and treated castrates, without a pronounced difference being visible. In the 2 adult groups, both ventral prostates and seminal vesicles were typically castrate in type. Thus there is no definite suggestion of an effect of cortisone upon these castrate end-organs.

F. *Spayed females:* Two groups of females, ovariectomized as adults and treated with 5.0 mg. of cortisone daily for ten days (9 treated individuals), had uteri which showed reductions in weight as compared with those of the controls of 1 per cent and 8 per cent; histologic study did not indicate a detectable difference from controls, hence no uterine stimulation was suggested by these studies.

This series of animals, therefore, fails to demonstrate an effect of

TABLE 3. EFFECT OF DAILY SUBCUTANEOUS INJECTIONS OF ACTH IN RATS

No. of animals treated	Treatment		Age at autopsy	Av. body wt. of controls (Gm.)	Percentage change in treated animals						
	No. of days	Daily dose (mg.)			Body wt.	Adrenals	Testes	Ventral prostate	Seminal vesicles	Ovary	Uterus
				A. *Intact males*							
2	12	1.25	24 da.	23	+26	−24	− 8	−24	−12		
3	12	1.25	29 da.	60	−15	+ 7	−18	−12	− 4		
2	9	2.50[a]	37 da.	105	0	+21	−14	+11	−25		
3	9	5.00	48 da.	109	−20	+33	+13	−20	−33		
2	7	1.00	4 mos	231	− 7	− 8	+20	+ 8	−12		
2	14	1.00	4 mos	200	+23	+ 2	+10	+ 8	+11		
4	10	5.00	adult	286	−13	+38	+15	+31	+12		
				B. *Castrated males*							
3	9	2.50	21 da.	40	− 7	−17		−14	− 8		
3	9	2.50[a]	24 da.	44	0	+ 8		−16	−14		
2	12	5.00[b]	28 da.	52	− 7	0		− 2	+15		
2	11	2.50	33 da.	86	−19	+35		+56	+ 5		
3	13	5.00[b]	40 da.	88	+ 4	+25		+36	− 6		
3	11	5.00	44 da.	80	+30	−17		+23	− 5		
3	10	5.00	adult	317	− 4	+ 2		−21	− 3		
6	10	5.00	adult	339	−11	+20		− 3	+ 3		
				C. *Intact females*							
3	12	1.25	24 da.	45	− 6	+26				+ 7	+ 3
3	9	2.50	24 da.	38	+ 2	+ 3				−10	− 8
3	9	5.00	48 da.	105	0	−13				−18	−48
2	7	3.00	55 da.	110	+19	−14				+ 3	− 9
2	14	3.00	62 da.	141	− 8	− 2				− 1	−45
3	7	5.00	83 da.	139	− 4	0				−12	−32
4	10	5.00	adult	238	− 3	+18				+42	+16
				D. *Spayed females*							
3	11	5.00	44 da.	77	+24	− 4					− 5
3	10	5.00	adult	241	+ 1	−13					+ 9
6	10	5.00	adult	234	− 9	+22					−11

a (3rd col) =sample 84-5 HSI, 1 daily injection.
b (3rd col) =sample 84-5 HSI, 2 daily injections.

cortisone on the accessory reproductive glands of castrated or intact males or females, of either young or older ages. Gonadal changes in relative weight or histology were indefinite in the male, but in the female the ovaries were consistently heavier in the treated animals. In treated prepuberal females there were larger numbers of follicles showing antra, and these were somewhat larger cavities than in follicles from control animals; ovulation had not occurred up to the 36th day of age. In adult ovaries the same tendency was evident and corpora lutea were present, but not visibly different in treated animals and controls. These ovarian weight increases were not accompanied by perceptible uterine weight increases, hence hormonal secretions were not detectably modified.

IV. TREATMENT WITH ADRENOCORTICOTROPIC HORMONE (ACTH)

In an attempt to explore possible effects that ACTH might exert on reproductive structures in the rat, 23 different groups of animals were treated daily by single subcutaneous injections of the hormone, and 2 litters with two daily injections, for periods ranging from seven to twenty days; each group was compared with its own appropriate controls. Autopsy ages of 20 days up to 85 days involved groups of littermates; groups subjected to autopsy at older ages consisted of entirely comparable animals, although not littermates in all cases. Among the 25 groups containing 150 animals, 75 were subjected to treatment and 75 served as controls. Treated animals consisted of A) normal males, B) castrated males, C) normal females, and D) spayed females, different groups being sacrificed at ages ranging from 21-day-old young rats to unknown-aged adults weighing more than 300 grams.

Recorded in Table 3 is a summary of body weight, adrenal weight and reproductive organ weight changes in the treated animals in comparison with the appropriate controls. Fresh organ weights were dealt with as in Table 2, comparisons being made between the weight of organs in milligrams per 100 grams of body weight. The hormone utilized (Armour & Co.'s ACTH, sample 74 AX, 1 mg. total solids, equivalent to 0.5 mg. standard ACTH) was dissolved in saline in an amount sufficient for one week of injection; total daily doses were divided into separate quantities that were maintained in the frozen state until used. The original sample, maintained in the dry state under refrigeration until prepared for injection, was used for treatment in all groups but 4, which are designated in the table and receive special mention subsequently; these 4 groups were treated with a specially purified sample (Armour & Co., lot 84–5 HSI, 104 per cent potency in terms of standard).

A. *Body weight:* An examination of the column in Table 3, recording changes in the average body weight at autopsy of treated animals of all 4 sexual categories in terms of their respective controls, reveals that of the 25 litters (or groups) no change was noted in 3, increases of from 1 to 26 per cent occurred in 8 groups, and decreases of from 3 to 19 per cent were present in 13 groups. If one arbitrarily eliminates changes of 10 per cent or less as insignificant, one finds that increases in body weight of treated animals occurred in 5 groups and reductions in 5. Since increases, as well as decreases, are recorded in both young and adult groups, after treatments with low and high dosages, and in animals representing different sexual states, these data fail to indicate a positive trend in relation to a definite effect of ACTH in dosages used on body weights over periods of treatment ranging from seven to fourteen days. A larger number of animals, of similar age, treated for the same length of time, and in similar sexual states would be needed to determine whether ACTH exercised a definite effect on the body weight of treated animals. The design of the present experiment, with its many variables, does not provide suitable conditions on this point. An examination of the previously reported data of Asling, Reinhart and Li (24) showed a retardation in the growth weight of

young treated males (5 males injected daily from day 22 to day 60—two to three intraperitoneal injections daily of 2 mg. or 3 mg. of a highly purified preparation). After treatment for twenty days, experimental males were 38 per cent lower in body weight than were controls and after forty days there was a reduction of approximately 60 per cent.

B. *Adrenal weight:* The seventh column in Table 3 reveals that in some groups the converted adrenal weights in ACTH-treated animals were slightly heavier than those from the respective controls, a result which has been found generally. It is to be remembered that data recorded here are expressed in terms of converted weights.

A few conspicuous reductions in the weight of the adrenal of treated animals may be pointed out as follows: 24 per cent reduction after twelve daily injections of 1.25 mg. daily into animals prior to autopsy on day 24; 17 per cent reduction in castrated males 21 days and 44 days of age following injection for nine and eleven days with 2.5 mg. and 5.0 mg., respectively; and similar reductions in 2 groups of normal females at autopsy ages of 48 and 55 days, and in 1 group of spayed adult females.

C. *Gonad weight:* Among 7 groups of intact males subjected to autopsy at ages ranging from 24 days to adulthood, including 18 treated males, reductions in fresh weight of the testes above 10 per cent occurred in two litters killed on day 37 or earlier, whereas in animals killed at ages older than 40 days, 4 groups showed an increase in testis weight of from 10 to 20 per cent above that of the normal controls. These increases followed daily treatments with from 1 mg. to 5 mg. of ACTH, and they were not proportional to dosage. The effects are therefore not considered to be of significance.

Modifications of ovarian weights from treatment of 7 different groups of intact females were unimpressive except for 2 groups. In one litter composed of 3 each of treated and control females (nine injections, 5.0 mg./day) sacrificed on day 48, average converted ovarian weights showed a reduction compared with controls, of 18 per cent; and in a group of adults, 4 treated females showed an increase of 42 per cent in ovarian weight. A histologic study of ovaries has failed to reveal any distinct modification of the tissue in a qualitative sense by treatments with ACTH. Weight differences mentioned here appear to depend entirely on differences in the number and size of normal-appearing corpora lutea present in the ovaries.

D. *Hormone secretion:* An attempt was made to determine whether treatments with ACTH modified in any manner the secretion of hormones by intact gonads, through a comparison of fresh weights of target organs and by a histologic study of these organs (in males, ventral prostate gland and seminal vesicles; and in females, uterus).

Fresh weights of ventral prostates and seminal vesicles of ACTH-treated intact males showed some variation from those of the controls of the group but no consistent trend became evident. In 2 cases the same litter or group showed increases in prostate weight and decreases in seminal vesicle weight; 3 groups showed decreases and 2 groups showed increases in both organs. This suggests ordinary individual variability rather than definite responses to a modification of hormone secretion. Younger aged males could reveal precocious or excessive testis hormone secretion, since seminal vesicle epithelium does not show a high columnar epithelium or secretion granules until an age of 45 to 50 days. Inhibition of testis secretion could be easily detected from a low and inactive seminal vesicle epithelium in 60-day-old to adult animals. Histologic preparations from these experiments failed to suggest either stimulation or depression of target organs, hence yielded no evidence that treatment with ACTH in any manner modified testis hormone secretion.

In similar comparisons no evidence emerged to suggest a modification of ovarian

secretion by treatment with ACTH. Uterine weights and general histologic structure naturally vary in ordinary cycles but such minor variations as appeared were neither pronounced nor consistent.

E. *Castrated males:* Particular attention was directed to a study of the ventral prostate and seminal vesicles of castrated males treated with ACTH, and especially treated prepuberal males.

It may be recalled that in mice (25–27) and to some extent in rats (28, 29) the x-zone of the adrenal has been associated with the sexual condition of the animal, and by some the x-zone has been considered the source of the adrenal's capacity to secrete androgens and estrogens. Price (8) pointed out that male rats castrated at birth showed good subsequent, although brief, development of the ventral prostate, suggesting some source of androgens in the organism outside the testis. Full secretory development in the prostate occurred and was maintained until an age of approximately 30 to 35 days, when it began a recession towards the castrate level and so remained. In keeping with evidence available it was suggested that an early stage of adrenal development provided androgens that were no longer available when recession of the x-zone occurred. Burrill and Greene (30, 31) studied the prostates from young castrated males, comparing these with the same tissues recovered from castrate+adrenalectomized males, and supported the notion of an adrenal source of androgens. Davidson and Moon (32) and Davidson (33) claimed that the treatment of castrated prepuberal rats with adrenocorticotropic hormone induced much greater development of prostates and seminal vesicles, suggesting that the adrenal was stimulated to produce greater amounts of androgen.

During the course of the experiments herein reported the treatment of intact males, young or adult, with ACTH failed to yield data suggesting that additional androgens were secreted by the adrenals of intact males; therefore, it became of particular interest to study the effects of ACTH treatment in castrated males. In Table 3 B a summary of results is recorded for 6 litters subjected to autopsy on day 44 or earlier, following a short treatment with ACTH, and for 2 adult groups. The young males were injected immediately following castration but in the older 2 groups ACTH treatments were not begun until sixty days or longer after castration.

Since treatment of intact males, as well as of 3 litters of castrated males sacrificed at ages of 21 days, 33 days, and 44 days (up to 5.0 mg./day of ACTH) failed to show definite effects from treatment with the original sample used, a specially purified sample of ACTH was obtained for final tests. Through the courtesy of Dr. L. L. Lachat of Armour & Co., the specially purified sample (lot 84–5 HSI, assayed as 104 per cent potency) was utilized for treatment of castrated young in 3 additional litters sacrificed on day 24 (nine single subcutaneous injections, 2.5 mg./day), on day 28 (twelve daily treatments, 5.0 mg./day in two subcutaneous injections) and on day 40 (thirteen daily treatments, 5.0 mg./day in two subcutaneous injections); these 3 litters are designated a and b, in Table 3 under the column on the daily dose.

The data for these 3 litters show that autopsy on day 24 yielded weight decreases of 16 and 14 per cent, respectively, for ventral prostates and seminal vesicles, autopsy on day 28 a reduction of 2 per cent for ventral prostates but an increase of 15 per cent for seminal vesicles, and autopsy on day 40 an increase in prostate weight of 36 per cent but a decrease of 6 per cent for seminal vesicles. It should be pointed out that the 36 per cent increase in prostate weight involved an actual average weight difference of 2.1 mg. Although dissection of fresh tissues was accomplished under a binocular dissecting microscope, slight variations in clearing away fat and connective tissue are inevitable and some question may well be raised as to the value of very minor original weight

differences. The weight data as they stand in the table suggest increases in fresh weight of the ventral prostate after ACTH treatment (with autopsies at ages 33 to 44 days) without similar increases for seminal vesicle weights, but decreases in prostate weights in treated males sacrificed at earlier ages.

Histologic study of these two target organs is believed within limits to be of greater importance in detecting androgen secretion from possible stimulation of the intact adrenals, than is a study of the fresh weights of tissues, unless these latter show consistent and quite convincing differences. The ventral prostate and seminal vesicle histologic picture in animals treated with the original sample was entirely similar to that in their respective controls; hence evidence to indicate an increased androgen secretion under treatment was lacking.

Turning attention to the target organs of animals treated with the special sample (lot 84–5 HSI), Figures 8 and 9 show sections through the ventral prostates of a castrated control and of a treated animal in the litter sacrificed on day 24. The prostate of the untreated castrated control (Fig. 8) consisted of well developed acini lined with an epithelium in a good secretory condition. Light areas, not well shown in the figure, were present in the epithelium of many acini and the gland was typical for the early castrated male. Figure 9 is from a male that received nine daily single doses of 2.5 mg. of ACTH just prior to autopsy. The tissue of the gland compares favorably in character with that of the castrated untreated male (Fig. 8) and no evident superiority has been gained from the treatment. Seminal vesicles of both the castrated control and the ACTH-treated animals were entirely similar in character and were typically castrate in type. In each animal of both the control and the treated members, one fails to find evidence of an androgenic effect subsequent to treatment with ACTH.

Tissues of the litter castrated on day 17 and sacrificed on day 28 (the treated members received twelve days of treatment with 5.0 mg./day administered in two doses) revealed that in the 3 castrated controls and in 2 ACTH-treated castrates the ventral prostate was in excellent condition. Acini were well developed with tall columnar epithelium and many weak, light areas were visible. The untreated castrate prostate in each of the 3 animals was typical for the age and was as strongly developed as the prostate from each of the 2 castrates subjected to ACTH treatments for the twelve preceding days. Seminal vesicles of the control and treated castrates showed the typical castrate picture. There was no indication of a stimulation from ACTH treatment of either ventral prostates or seminal vesicles.

One litter was especially selected for castration and ACTH treatment beginning on day 28, when the prostate is in a high secretory state, with autopsy on day 40 when prostate involution from castration is well under way. Three control castrates and 3 castrates subjected to treatment with ACTH for thirteen days (5.0 mg./day in two doses) were carefully compared. Treated animals showed body weight increases of 4 per cent above those of the untreated controls; ventral prostates were 36 per cent heavier and seminal vesicles 6 per cent lighter than in the untreated castrates. Histologic study revealed that the ventral prostates of the control and the treated animals were typically castrate and much inferior to those found in the litter sacrificed on day 28. Acinus epithelium was of approximate nuclear height; a few acini contained cells with slightly more cytoplasm, but in none of these animals were light areas visible in prostate epithelium. In general, the ventral prostates of both control and treated castrates were castrate in type and the ACTH treatment had failed to delay the characteristic prostate involution occurring over this period. Seminal vesicles were entirely castrate in type. Thus, ACTH treatment failed to prevent involution of the castrate ventral prostate or to stimulate seminal vesicles; no androgenic effect was detectable.

F. *Spayed females:* Ovariectomized females were treated with ACTH to determine whether secretion of estrogen-like substances, possibly from an adrenal source, could be detected. Among 3 groups, including 12 treated, ovariectomized females, the fresh weight of the uterus failed to indicate any appreciable effect from treatment with ACTH. In 1 group, the average uterine weight for 3 treated females receiving 5.0 mg. of ACTH daily for ten days was 9 per cent greater than for the untreated controls, whereas the other 2 groups of treated females showed reductions in the weight of the uterus of 5 and 11 per cent. Histologic examination likewise failed to reveal differences between the treated and untreated control uteri, hence no effect from the treatment became evident.

In summary, one is unimpressed by any responses of reproductive structures as a result of treatment with ACTH in the dosage range here employed. Changes in the weight or histologic picture of gonads or accessory organs in intact males and females were not clearly indicative of any effect from treatment; accessory reproductive structures failed to

TABLE 4. EFFECT OF BILATERAL ADRENALECTOMY IN MALE RATS. (BODY WEIGHT IN GM. INTERNAL ORGAN WEIGHTS IN MG. PER 100 GM. OF BODY WEIGHT)

Group	No. of animals	Treatment	Av. body wt. at autopsy (Gm.)	Testes		Vent. prostate		Seminal vesicles	
				Wt. (mg.)	% change	Wt. (mg.)	% change	Wt. (mg.)	% change
		I. *Adrenalectomy day 50; autopsy day 65*							
A.	5	Normal intact controls	168	1,280		75.1		122	
B.	5	Intact +DCA 100 μg.	178	1,250	−2	60.5	−19	102	−16
C.	5	Adrenalectomy +DCA 100 μg.	160	1,170	−8	54.0	−28	65	−46
D.	5	Castrated control	162			7.4		7	
E.	5	Castration +DCA 100 μg.	150			6.2	−16	6	− 8
F	4	Castration +adrenalectomy +DCA 100 μg.	150			7.0	− 5	12	+65
		II. *Castration +adrenalectomy day 20; autopsy day 29*							
	3	Castrated control	55			15.0		5.1	
	3	Castration +adrenalectomy	34			14.5	− 3	7.7	+50
		III. *Castration +adrenalectomy day 21; autopsy day 31*							
	1	Castrated control	50			12.0		6.4	
	2	Castration +adrenalectomy	46			8.8	−26	7.3	+14
	2	Castration +adrenalectomy +DCA 50 μg.	55			7.4	−38	6.1	− 4
		IV. *Castration +adrenalectomy day 21; autopsy day 34*							
	2	Castrated control	71			7.7		4.2	
	2	Castration +adrenalectomy +DCA 100 μg.	71			5.3	−31	5.4	+12
		V. *Castration +adrenalectomy day 25; autopsy day 37*							
	4	Castrated control	69			9.3		3.8	
	3	Castration +adrenalectomy	57			9.3	0	5.8	+52

show any systematic modification in intact animals; and treatment of castrated males or females failed to reveal changes in the accessory reproductive organs that in any manner suggested a stimulation of the adrenal gland to secrete sex hormone-like substances.

V. ADRENALECTOMY AND THE REPRODUCTIVE SYSTEM

A limited study was made of the possible effects of adrenalectomy upon the male reproductive system; the experiments have been confined to periods of short duration and the animals utilized, along with some data on weights of the accessory organs, are listed in Table 4. Older males treated from day 50, with autopsy on day 65, were employed to study effects of adrenalectomy as the animals approached reproductive maturity, and younger prepuberal males constituted a group for the examination of "andromimetic" effects upon accessory reproductive organs.

Adrenal glands were removed through two dorso-lateral incisions, and castration was performed at the same time through a low midventral incision. All adrenalectomized animals had 2 per cent saline solution available and some groups received daily subcutaneous injections of desoxycorticosterone acetate. Younger males were usually given one subcutaneous injection of 1.25 mg. of cortisone on the day of operation.

Reproductive maturity

For the purpose of an initial experiment involving animals of similar age a shipment of young males was obtained from a commercial breeder (Abrams). When these males attained the age of 50 days they were allocated to 6 groups of 5 males each and treated as follows (group I ,Table 4): A) intact, untreated controls, B) intact, treated daily with DCA, C) adrenalectomized, treated daily with DCA, D) castrated controls, E) castrated, treated daily with DCA, F) castrated, adrenalectomized, treated daily with DCA. At the end of fifteen days 29 animals were subjected to autopsy, 1 male in group F having died.

Table 4 (I) reveals that weights of testes were unaffected by either the daily treatments with DCA, or by adrenalectomy+DCA. The appearance of the fresh organs was similar in all groups, and spermatozoa from the epididymis became motile when suspended in saline solution. Percentage changes in testis weight in animals treated with DCA were unimpressive. The ventral prostate glands and seminal vesicles of intact males treated with DCA, and adrenalectomized animals treated with DCA, showed slight average reductions in fresh weights of these target organs. Histologic study, however, revealed that in each group both the ventral prostates and seminal vesicles displayed a normal, high columnar, secretory epithelium. Since no detectable difference was noted between the groups with intact testes, it is assumed that DCA alone did not exert an appreciable effect upon the testes (spermatogenetically or hormonally), nor did adrenalectomy accompanied by DCA treatments show either a stimulating or depressing effect on spermatogenesis, or upon hormone secretion, so far as could be revealed by histologic differences in the target organs.

Turning attention to the 3 castrate groups (D, E, F), treatment with DCA alone

was accompanied by a reduction in weight of the ventral prostate of 16 per cent (1.2 mg.) in comparison with that of the untreated castrates, and weights of seminal vesicles were reduced by 8 per cent. In adrenalectomized castrated rats treated with DCA a smaller weight reduction was noted in ventral prostates and a sizable increase in the weight of the seminal vesicles (4.9 mg. or 65 per cent). Histologic study again failed to bring forth detectable differences in either ventral prostates or seminal vesicles in the control and treated castrates. In all cases, acinar epithelium of both prostates and seminal vesicles was typically castrate in type and tissues from ordinary castrates could not be distinguished from those of adrenalectomized castrates.

Thus the limited utilization of DCA in the dosage employed failed to exert any appreciable modification on reproductive structures. Testes were unchanged in weight or structure. Whereas slight reductions occurred in fresh weights of ventral prostates and seminal vesicles (19 per cent and 16 per cent, respectively) there was no detectable modification in the histologic picture of these organs from treated males, in comparison with those from untreated controls; control and treated males demonstrated a high secretory state in these end-organs. The combination of adrenalectomy and treatment with DCA caused no detectable modification of the testis, aside from a reduction in average weight of 8 per cent. Histologically the organs were normal and epididymal spermatozoa showed motility. The prostate and seminal vesicles were reduced in proportionate weight more than with DCA alone (ventral prostate 28 per cent, seminal vesicles 46 per cent below weights in normal group). Histologically, however, both these glands were found to be in a high secretory state and did not deviate appreciably from the condition found in normal control animals. In castrated males treated with DCA the average weights of the accessories showed a slight reduction over those of untreated castrates (ventral prostate 1.2 mg. or 16 per cent; seminal vesicles, 1 mg. or 8 per cent), but histologically no detectable differences were found. The involuted condition of the epithelial lining of these two end-organs in treated castrates speaks against any degree of stimulation of the accessory organs by DCA. In castrated males adrenalectomy combined with DCA treatments was followed by a 5 per cent reduction in the fresh weight of ventral prostates but by a 65 per cent increase in the weight of the seminal vesicles (compared with untreated castrates). Histologically, the glands in the treated and untreated castrates were not demonstrably different; the epithelial lining of the acini was castrate in type in all cases. Within the limits of these experiments, therefore, treatment with DCA or adrenalectomy, or combinations of both, failed to reveal appreciable modifications in the male rat just attaining sexual maturity, either in the state of the testis, gametogenetically and hormonally, or of the accessory organs of the castrated males.

Prepuberal adrenalectomy

Price (8) demonstrated that ventral prostates in rats castrated shortly after birth developed into secretory glands until about days 30–35, following which epithelial involution occurred and the gland became castrate in type. The suggestion that young adrenals might serve as an extra-gonadal source of androgens for a short period after birth, accounting for the unexpected secretory differentiation (28), received some support from the experiments of Burrill and Greene (30, 31) who presented evidence that the accessory glands in young castrated and adrenalectomized male rats showed greater involution than in those castrated but not adrenalectomized.

The males from 4 litters of colony bred rats were utilized to study the effects of ad-

renalectomy along with castration. These litters, consisting of castrated controls and castrated adrenalectomized males designated groups II to V in Table 4, were operated on on days 20, 21 (2 litters) and 25, with autopsy on days 29, 31, 34 and 37.

Group II: Three males castrated on day 20 served as controls for 3 littermates castrated and adrenalectomized on the same day, all being sacrificed on day 29. The adrenalectomized castrates received a single injection of 1.25 mg. of cortisone on the day of operation, and there was available at all times, 2 per cent saline, the ordinary laboratory diet of Purina checker squares and fresh lettuce. Average body weight at autopsy showed a reduction of 38 per cent in the adrenalectomized males in comparison with castrated controls, and the average weights of ventral prostates were 40 per cent lower in adrenalectomized animals. Conversions of actual weights to mg./100 Gm. of body weight gave for ventral prostate the value of 3 per cent reduction, entered in the table. Actual weights for seminal vesicles were approximately the same in the two groups but conversion to percentage body weight showed the seminal vesicles in adrenalectomized animals to be 50 per cent greater in weight than the average for ordinary castrated controls.

Histologic study of the ventral prostates demonstrated those of the ordinary castrate to be in excellent secretory condition, with large acinar lumina and tall columnar epithelial cells; however, light areas of secretory cells were extremely weak or absent. It is usual at this age to find the ventral prostates in good secretory condition, since at the age of 30 to 35 days involution ordinarily occurs, although there is some variation in this process with age of animal. Figure 10 is a photomicrograph of a section from the ventral prostate of a castrated male and it is to be compared with that from a littermate subjected to simultaneous castration and adrenalectomy (Fig. 11); in this group, no adrenal rests were disclosed at autopsy. The prostate tissue of these 2 littermates is closely similar in character and that from the castrated adrenalectomized rats is far from a completely involuted type. As one scans sections throughout the gland it becomes apparent that on the whole the prostates from ordinary castrates are somewhat better developed than the prostates from the castrated adrenalectomized group. Involution on the whole is less marked; acini are in general larger, with more acini provided with high columnar epithelium. Whether the somewhat greater involution of the adrenalectomized castrate is due directly to loss of some substances provided by the intact adrenal, or is due to the general reduction of well being indicated by a 38 per cent body weight reduction, remains a question. The condition of the seminal vesicles is irrelevant to the problem, since in the case of both groups of animals a basic underdeveloped condition prevailed. It is to be noted that little claim has been advanced to suggest that so-called andromimetic adrenal effects extend to the seminal vesicles.

In this group, therefore, it is to be emphasized that ventral prostates in the absence of the testes and adrenal glands were in a good secretory state, but that they were slightly inferior to those from castrated animals with adrenals intact.

Group III: This litter contained 6 males operated on on day 21, 2 of which served as castrated controls (1 died prior to autopsy), 4 being castrated and adrenalectomized. Two of the latter received daily subcutaneous injections of 50 μg. of DCA. All adrenalectomized males received a single subcutaneous injection of 1.25 mg. of cortisone on the day of operation. At autopsy on day 31 the average body weights were relatively close, the lowest weights being recorded for the untreated adrenalectomized pair; 1 of these latter had a small adrenal rest (0.2 mg.). Converted fresh weights revealed reductions of 26 per cent in prostate weight for untreated adrenalectomized males and 38 per cent reduction for those receiving DCA. Seminal vesicle weights were, respectively,

14 per cent above and 4 per cent below that of the single castrated control.

On histologic study the castrate prostates were more involuted than those of the 29-day autopsy series (group II). In general the acini were smaller in size and cytoplasmic reduction in the secretory cells was pronounced. The epithelium of many acini was reduced essentially to nuclear height, with some showing small amounts of cytoplasm between the nucleus and the lumen end of the cell. Although involution had not reached the typical castrate level, it was far advanced. The prostates of adrenalectomized castrates, with or without treatment with DCA, differed in no material way from those of the castrated control. Seminal vesicles from all animals of the group were typically castrate and could not be identified except by number on the preparation.

Thus, in this litter, castrated and adrenalectomized on day 21, at a stage when the ventral prostate would have been in a high secretory state, this organ at autopsy on day 31 was as well preserved as in the simple castrate; but in all cases involution was pronounced.

Group IV: This litter contained 5 males, all of which were castrated on day 21 and 3 of which were adrenalectomized on the same day; at autopsy on day 34 one of the latter had a relatively large adrenal rest on one side and was discarded from further consideration. Each adrenalectomized animal on the day of operation received a single subcutaneous injection of 0.5 mg. of cortisone, and daily thereafter received 100 μg. of DCA. At autopsy on day 34 the average body weights of the 2 castrated controls and 2 adrenalectomized animals were the same. Converted weights of fresh prostates from adrenalectomized castrated males were 31 per cent below those of the castrated controls, but seminal vesicle weights were 12 per cent higher.

Histologic sections showed that the prostates of the castrated controls were in a better secretory state than was the prostate at autopsy of the castrated control on day 31 (group III). This is not surprising, since the time of involution of prostates in castrated controls of various litters varied within rather narrow limits. Figure 12 represents the prostate gland from the 34-day-old castrated control, and involution can be seen to have gone further than in the castrated control of day 29 (Fig. 10). The acini of the 34-day-old castrate have well developed lumina and many of them show secretory epithelium in the low-columnar stage, with cytoplasm approximately twice the nuclear height. No light areas in epithelial cells are seen. Figure 13 shows sections through the prostatic acini of the prostate in a castrated adrenalectomized animal and it is evident that these acini have well distended lumina and a fair retention of cytoplasm within the epithelial cells (compare with Fig. 12). On the whole, however, the prostate in the castrated adrenalectomized animal had fewer large acini than in the castrated controls and one gained the distinct impression that on the whole the prostates of adrenalectomized castrated animals were somewhat inferior to those of the castrated controls. Again, it is to be emphasized that adrenalectomy did not result in a typical castrate prostate, and that the apparently somewhat greater involution was of relatively small proportions. The changes here were minor rather than pronounced.

In this litter, therefore, the impression was gained that castration plus adrenal removal had been accompanied by somewhat greater involution of the ventral prostate than in the castrated control.

Group V: This litter contained 8 males, all of which were castrated on day 25 and 4 of which were also adrenalectomized on the same day (1 died prior to autopsy). Each adrenalectomized animal received on the day of operation a single injection of 2.5 mg. of cortisone and on the second day, 1.25 mg. of cortisone. No other treatment was provided except 2 per cent saline for drinking. At autopsy on day 37 the actual weights

of the ventral prostates were 20 per cent lower in the adrenalectomized animals but on conversion to mg./100 Gm. of body weight, prostate weight averages were the same; thus, in Table 4 the change in treated prostates is designated zero. Seminal vesicle weights were slightly heavier in adrenalectomized animals, and upon conversion became 52 per cent heavier than those from castrated controls.

Histologically the prostates of castrated controls had not undergone complete castration involution. The epithelial lining of the acini for the most part approached the castrate condition, with some epithelium having cytoplasm extending slightly beyond the nucleus. The prostate in the adrenalectomized castrated animal was not perceptibly different from that in the ordinary castrated control, hence no apparent effect of adrenal removal was evident. Seminal vesicles of all animals of this group were typically castrate in type and indistinguishable among themselves.

VI. DISCUSSION

The possible interrelationship between the function of the adrenal gland under relatively normal conditions, and the reproductive system, is an interesting and important problem. Parkes (1) has reviewed a great deal of the literature bearing on the field. The oldest and perhaps strongest evidence for an effect of adrenal secretions upon the reproductive system or general sexual characters is associated with pathologic conditions such as adrenal tumors in the human individual, particularly in women. More recently, excellent corroborative evidence from experimental animals has been added through the studies of Wooley and Little (4–7). They subjected certain strains of mice to gonadectomy shortly after birth. Within a few months there developed a nodular hyperplasia in the adrenal cortex that sometimes metastasized as carcinoma, accompanied by activity of the reproductive system quite comparable to that in the normal intact animal. This condition, although demonstrated for certain strains of mice, does not apply to many other strains or to other species so far as is known at present. Whereas it appears well demonstrated that the adrenal pathology is associated with the production of androgen-like and estrogen-like substances the question still remains unanswered whether under normal conditions the adrenal contributes such hormones in effective amounts.

Various extracts of adrenal tissues have been tested for effects on the reproductive tissues. Parkes (34) has reported androgenic effects from inunction on the comb of chickens of crude extracts of horse adrenals, as well as from highly concentrated discard fractions of pig adrenals. On the other hand, Connor (35) determined that cortical preparations exhibiting life-saving effects were without effect upon the reproductive system of the immature rat; Gaunt and Parkins (36) used potent adrenal extracts but could not elicit responses from the reproductive tracts of young rats or young chickens; Howard and Grollman (37) used relatively pure extracts of adrenal cortex and failed to find an effect on sexual maturity in the rat. Carnes (38) reported that extracts of human fetal and newborn

adrenals placed on the comb of day-old chicks or injected into immature rats failed to elicit responses characteristic of sex hormones; Gersh and Grollman (39) utilized extracts of fetal pig adrenal cortex, fetal and newborn human cortex, and adrenals from x-zone-bearing mice without being able to detect an androgenic effect. Wolff and colleagues (40) showed that chick coelomic transplants into a two- or three-day-old embryo, with autopsy from the fourteenth to sixteenth days, had no masculinizing or feminizing action on the gonads or ducts of the host.

Transplantation of adrenal gland tissue has been carried out, using such localities for graft sites as the ovary (41), abdominal musculature (42), anterior chamber of the eye (43), ovarian area (44), brain (45), external ear and perirenal localities (46), with life-maintaining functions demonstrated in some cases; but in none has evidence been advanced for an androgenic effect.

It is to be recalled that Katsch, Gordon and Charripper (47) made autotransplants of adrenals to the seminal vesicles of adult males, removing the testes two to three days later, and reported that the involution of the seminal vesicles as a result of castration was somewhat retarded when compared with that in castrated animals receiving transplants of muscle tissue rather than of adrenal tissue. Their criterion of an effect from the adrenal graft was based upon comparative weights of seminal vesicles removed ten, twenty and thirty days after transplantation, and upon histologic study. Although making it clear that the adrenal graft located in close proximity to seminal vesicle acini failed to maintain the secretory character of seminal vesicle epithelium, they concluded that "adrenal cortex displays androgenic capacity under these conditions. . . . " More convincing evidence for androgen secretion would have been the repair of castrate epithelium to a secretory state.

Observations concerning androgen secretion by transplanted tissues

The seminal vesicle of the castrated rat is in some respects a favorable location for transplantation of living tissues, to test for androgen secretion. Moore, Hughes and Gallagher (20) demonstrated not only the sharp cellular changes in the secretory epithelium of this organ that are associated with the presence or absence of androgens, but also the speed with which the cellular changes are modified by hormonal manipulations. There is an added advantage that in recovery of transplanted tissues the detector organ can be recovered along with the graft. Advantage has been taken of that condition by Katsch and colleagues (47) and by Jost (18).

The utilization of the castrate seminal vesicle as a location for transplanted testes from the rat embryo has convincingly demonstrated the capacity of near-term fetal testes to secrete androgens, to liberate these

secretions locally about the graft, and to stimulate the secretory epithelium in acini located close to the testes graft (Jost (18), and present experiments). It is suggested that the hormonal substances pass to contiguous tissues by diffusion, rather than through the general circulation. Jost demonstrated, and experiments reported herein confirm, that acini in the opposite seminal vesicle in which no functional testis graft is present fail to reveal any androgenic stimulation, whereas those contiguous to the testis graft are in a high secretory state. The hormonal effect is entirely local, since acini in the same seminal vesicle located a short distance away from the graft are typically castrate in type. Secretions produced in the graft diffuse into the tissues and exert a stimulating effect on the target organ within a period of from six to ten days after transplantation (Figs. 1, 2 and 5). This fact, however, proves only that the rat testis at birth is capable of secreting androgens under special conditions, and cannot be taken as proof that the intact testis of the fetus is secreting hormones, although it is possible that it may be secreting hormones in very small amounts. Jost and Colonge (19) emphasize that the activity of the host pituitary is important for the precocious stimulation of androgen secretion by the testis grafts; in the hypophysectomized castrated host, testis grafts failed to stimulate acinar epithelium.

The proof of the quick responsiveness of the castrate seminal vesicles to hormones secreted by testis grafts immediately suggests proper means to determine whether adrenal tissues secrete androgens. In experiments reported here, none of the 12 adrenal grafts recovered from fourteen to sixteen days after transplanatation gave evidence of having secreted androgenic substances. Acini located immediately next to good adrenal grafts were castrate in type. The grafts themselves were rated as fair-to-excellent and contained either cortical tissues alone or cortex surrounding a distinct medulla (Fig. 7). Thus the evidence suggests that the adrenal grafts were not secreting androgens.

Treatment with cortisone

Cortisone represents a powerful and important substance obtained from extraction of adrenal glands, and tests were made to determine whether it exerted definite effects upon the reproductive system. Since cortisone causes a marked reduction in the total weight of the organism, especially in the young growing animal, it appeared especially important to utilize the percentage of body weight in making comparisons between the gonads and accessory reproductive organs of treated animals and their littermate controls. Antopol (48) used sufficiently high dosages of cortisone in immature mice (1.25 mg. per day) and in mature mice (2.5 mg. per day) to cause death in many treated animals from the fifth to the twentieth day

of treatment. He reports for immature mice that gonads and accessory organs were below normal weight in treated males and females with suppression of spermatogenesis in adult males. Winter, Silbert and Stoerk (49) found that 3 mg. of cortisone daily for six weeks in 200-gram rats did not affect the testis, and the decline in weight of accessory organs was less than that for body weight; in castrated animals the seminal vesicles and the prostate were not stimulated. Ingle (21) used 5 mg. of cortisone per day, without modification of testes or accessory organs in adult rats.

In the present experiments, with fresh weights of organs expressed as milligrams per cent, a slight tendency to increases in testis weight appeared after treatment, in the higher dosages employed, but such an effect was by no means marked. Histologic study failed to reveal changes in testicular tissue as a result of treatment. Hormone secretion by the testes was not perceptibly modified by cortisone treatment, as judged by fresh weight or the histologic condition of the ventral prostate gland and seminal vesicles. Precocious hormone secretion in young males was not evident, since fresh weights of ventral prostates were frequently below those of untreated controls and in some cases were accompanied by slight increases in the weight of seminal vesicles; differences in the recorded weights are believed to be inconsequential. The results with the different dosages and the different age groups utilized for treatment, failed to suggest any consistent trend in effects. Histologic study failed to yield suggestions of either a stimulating or a depressing effect upon androgen secretion when dosages up to 5 mg. per day were employed. Adult castrated males treated with this substance failed to show evidence of stimulation of accessory organs, and histologically the target organs remained in a castrate state.

Normal intact females, receiving treatment with cortisone, rather consistently showed increases in fresh ovarian weight in comparison with controls. Histologic examination suggests that such weight increases rested upon a greater number of follicles with antra, as well as upon the greater size of the antra. In litters in which normal control ovaries had not yet ovulated, corpora lutea were likewise absent from treated individuals, and in older litters corpora lutea were present in both control and treated ovaries, with no obvious difference in these structures. Fresh weight of uteri did not vary perceptibly between treated and normal untreated females; hence there was lack of evidence for any modification in the rate of estrogen secretion in the treated females. Treatment with dosages up to 5 mg. per day in spayed females failed to modify fresh weights of the uteri. It therefore becomes apparent that in these experiments, treatment with cortisone in the several different age groups and sexual conditions failed to yield evidence for any definite effect of the substance upon reproductive structures.

Treatment with adrenocorticotropin (ACTH)

The stimulation of adrenal secretions by ACTH provides another means of studying the effect of the adrenal on the reproductive system; and since it was necessary to distinguish effects of increased adrenal secretions on the accessory reproductive organs separately from those mediated through gonadal activity, rats in the different sexual states were subjected to treatment. Variable dosages, ages and sexes, as well as intact and gonadectomized animals were employed for treatment with the same lot of ACTH.

a) *Intact animals:* Li and Evans (50) used ACTH preparations from sheep pituitary gland to treat male rats from day 26 to 56 (1 mg. daily) and reported depressed rates of growth for testes, seminal vesicles, prostate and Cowper's gland. Testes had somewhat smaller seminiferous tubules, with fewer of them containing sperm, but tubular injury or desquamation did not occur. In 89-day-old males treated for fifteen days (1 mg. daily) there was no effect from treatment. ACTH treatment of hypophysectomized males failed to maintain the weight of the testes or accessory organs. Baker, Schairer, Ingle and Li (51) used highly purified ACTH (1 mg. daily) in adult males for treatment over periods up to twenty-one days and reported a 13 per cent reduction in testis weight but no striking histologic changes; spermatogenesis was active and sperm abundant. Higher dosages were believed to reduce secretion of testis hormone, on the basis of a reduction in the number of Leydig cells, somewhat smaller seminal vesicles and certain histochemical changes in the tissues; coagulating glands of the prostate were little affected. Asling, Reinhardt and Li (24) injected 21-day-old males for a period of thirty-nine days, giving intraperitoneal injections of 2 or 3 mg. per day in two or three doses, and reported relative weight reductions of 33 per cent for testes, 65 per cent for seminal vesicles and 21 per cent for prostates. In comparison with hypophysectomized controls, hypophysectomized males treated with ACTH showed increases in weight of testes and accessories, but the authors suggest that the apparent genital stimulation was due to contamination of the preparation with a gonadotropin. Gassner *et al.* (52) reported that extracts of venous blood drawn from the adrenal sinus of cows duing the injection of large amounts of ACTH exerted an androgenic effect when placed on the comb of 3-day-old chicks, whereas ordinary peripheral blood exerted no such effect; they believe the androgenic action may represent a stimulation of the adrenal cortex to produce excessive androgens. Kitchell and Wells (53) could not obtain evidence for the secretion of androgens by fetal rat cortex. In general, therefore, little evidence has been presented to suggest a direct effect of ACTH upon gonadal activity, although one may find some evidence that gonadotropic activity of the

intact pituitary may be modified by the action of the ACTH on the intact pituitary.

In the present experiments, involving as they do a great range of variation in animal ages and dosages, pronounced effects upon the organs of the reproductive system have not been found. Testes of animals treated with ACTH showed a tendency to be slightly heavier than those from untreated controls when the time of autopsy was from day 50 to adult ages, but showed some decreases in weight in younger animals. However, the changes recorded are small and of doubtful significance. Histologic differences were not apparent, when treated and control testes were compared. Hormone secretion by the testis was not perceptibly modified by treatment, since neither the weights nor the histologic condition of ventral prostates and seminal vesicles exhibited modifications, in comparison with untreated control organs. Within the dosage range and age groups employed for study, therefore, one must conclude that treatment with ACTH failed to cause any definite modification in the male reproductive system of the intact rat. No consistent evidence was obtained to indicate a modification in androgen secretion, either by the intact testis itself, or by the adrenals. Intact females, similarly, were not definitely affected by treatment with ACTH. Fresh ovarian weight and structure, or uterine weight, were not modified in any consistent manner.

b) *Gonadectomized animals:* The lack of concrete evidence for increased androgen secretion during ACTH treatment of intact males, young or old, suggests that the testes are not stimulated by this substance to secrete excessive androgens. However, one might make the objection that evidence for the stimulation of androgen secretion by the adrenals after treatment with ACTH would be masked by the hormone effects from androgens secreted by the testes. It became important, therefore, to utilize castrated males for treatment. In an early series of treated adult castrates, as well as in 3 castrated litters subjected to autopsy on days 21, 33 and 44, convincing evidence was not obtained that adrenals had been stimulated to secrete androgens. Because of the positive claims by Davidson and Moon (32) and Davidson (33) that castrated prepuberal males show great stimulation from treatments with ACTH, the experiment was repeated on 3 litters sacrificed on days 24, 28 and 40, after treatments with a specially purified sample of ACTH, using up to 5 mg. per day administered in two doses.

On the basis of percentage of body weight, 1 of these litters displayed an increase in prostate weight of 36 per cent (2 litters in the earlier prepuberal series showed prostate increases of 56 and 23 per cent), whereas 2 of the litters showed decreases in prostate weight (3 of the earlier litters also showed decreases in prostate weight). The increases in prostate

weight were not regarded as significant, since the actual weight differences at autopsy were of the order of 1.3 to 3.7 mg. Furthermore, the seminal vesicles did not show an accompanying weight increase, but more frequently showed decreases. Whereas earlier studies in this laboratory on the responses of the end organs to androgens revealed a slightly lower threshold for prostate glands than for seminal vesicles in demonstrating histologic responses, later experiments of Price and Ortiz (11) on the weight sensitivity of these two organs showed that fresh-weight responses were always much greater for the seminal vesicles. Thus in autopsies at days 10, 14, 18, 26, 36, and 56 following six days of treatment with gonadotropins in order to stimulate testicular androgen secretion, or after direct daily injections of 0.1 mg. of testosterone propionate, in every case the increases in weight of the seminal vesicles were greater (100 to 500 per cent) than the increases in weight of the ventral prostates. The slight increases in ventral prostate weights in the present experiments, accompanied by a lack of response on the part of the seminal vesicle, can scarcely be regarded as sound evidence for excessive secretions from the adrenal as a result of treatment with ACTH.

It is thus clear that our inability to demonstrate androgen secretion by the adrenals as a result of ACTH treatment does not agree with the claims of Davidson and Moon. These authors (32) injected adrenocorticotropic extracts of sheep pituitary (whole) for nine days into rats that were castrated on day 21 and sacrificed on day 31. They report an average autopsy weight of accessory sex glands ("prostate, coagulatory gland and seminal vesicles") as 54 mg. for the 10 control castrates and 117 mg. for the 7 treated animals—or an increase from treatment of more than 100 per cent. Histologic evidence of stimulation of prostates consisted of larger tubules, more acini, and tall columnar epithelium with a clear supranuclear zone; in the castrates there were fewer acini, and cuboidal or low columnar epithelium. Seminal vesicle epithelium was slightly higher than that of controls. Davidson (33) presented additional material from males castrated on day 23, rested for five days, and then given nine days of treatment with 50 units of pituitary adrenotropic extract, with autopsy on day 38. Weights of castrate control accessories ("prostates and seminal vesicles") of 39 mg., compared with weights from treated animals of 56 mg., thus gave an increase of 43 per cent. It is not entirely clear just what tissues were weighed, since the average weight of castrate organs found by them at autopsy on day 31 was 54 mg., and on autopsy day 38 it was 39 mg., whereas combined weights of ventral prostates and seminal vesicles in our series of animals subjected to autopsy on days 28 to day 40 were in the range 9 to 13 mg. Davidson describes histologic effects as "marked repair of the prostate from the castrate level. The seminal vesicles showed little histologic change, but were definitely larger than in the control."

Nelson (54, abstract) reported that adrenocorticotropic hormone in immature male and female rats (normal, gonadectomized and others) induced enlargement of the sex accessories, and provided further evidence for accelerated production of sex hormones from the adrenals under the influence of ACTH. In the hamster (55) ACTH treatment did not demonstrate an andromimetic effect of the adrenals of castrate young males.

It appears probable that the adrenocorticotropin extracts employed by Davidson and Moon and by Nelson contained substances not present in the purified ACTH preparations of Armour and Co., but in the absence of more complete information, it is impossible to know the actual cause for the different results obtained. In experiments reported here, no convincing evidence was obtained to suggest that ACTH stimulated androgen secretion by the adrenals, or that the adrenal gland secreted androgens.

Adrenalectomy

The effect of adrenalectomy on the reproductive system presents essentially two problems, *i.e.*, possible effects brought on from operations on adults, and possible effects deriving from very early adrenalectomy, especially in males. Adrenalectomy during the adult stage of life has not been reported to show such effects as would suggest any participation of the adrenal secretion during such periods. Gaunt (56) found that testes of rats dying from adrenal insufficiency up to twenty-two days after operation showed no progressive signs of degeneration. In the few experiments reported in the present paper the writer was unable to note any pronounced effect upon the gonads or upon reproductive accessories that would suggest participation of the adrenal glands in the conditions found.

In the case of adrenalectomy during early postnatal stages in males a considerable amount of controversy has arisen and a brief review of the background as well as the results of investigation should be given.

The problem of androgen secretion by the adrenal gland during early postnatal life in rats and mice, especially in association with the transient x-zone of the adrenal, has been dealt with primarily by Howard (25–29), Price (8, 16) and by Burrill and Greene (30, 31). Price (8) demonstrated that the ventral prostate glands of rats castrated from four to six days after birth proceeded to develop into relatively advanced secretory stages that were maintained until approximately days 30 to 35, after which they began a typical castrate involution, to remain indefinitely at the castrate level. This has been confirmed by Burrill and Greene, by Howard, and by Gersh and Grollman (39). Price (16) further showed by transplantation that the effect depended upon the age of the host rather than upon age of the transplanted tissues. These findings provided suggestive evidence for an extra-testicular source of androgens during a limited period in early postnatal life. Masui and Tamura (57) described in mice a transitory zone

of the inner cortex of the adrenal gland, appearing about the third week, similar in males and females, with regression at approximate sexual maturity (pregnancy in females). The zone underwent hypertrophy in males after castration, thus suggesting some function associated with absence of testes. Howard (58) confirmed these relationships and found (2, 8) that the rat occasionally showed a somewhat comparable adrenal zone but less well defined, and that its presence coincided with the period in life when prostate development occurred in the castrated animal. She suggested that this x-zone was probably adromimetic and was possibly responsible for the secretory development of the prostate in the absence of testes. She furthermore studied details in castrated mice (26), correlated the state of secretory development of the prostate and seminal vesicles with the state of the x-zone, and while supporting the thesis of an andromimetic function of this tissue, was still loath to believe that all development suggestive of an extra-gonadal secretion of androgens could be attributed to the x-zone. In rats (29) she removed adrenals along with the gonads and concluded that the rat adrenal secreted physiologically appreciable amounts of androgen over limited periods, but in mice (27) she found that adrenalectomy was without appreciable effects on accessory organs; she holds that the adrenal cortex in this species does not secrete physiologically appreciable amounts of androgens, even during the stage of a well developed x-zone. Kitchell and Wells (53) report that adrenalectomy in fetal rats does not inhibit growth of reproductive organs in the male.

Burrill and Greene (30, 31) studied the ventral prostate of immature rats castrated on day 16 and adrenalectomized on days 21, 26, and 36 with autopsies on days 26, 36, and 31, in comparison with prostates from animals similarly castrated but with adrenals intact. Fresh weights of prostates from adrenalectomized castrated animals, in percentage of body weight ratios, showed reductions of 54 per cent on day 26, 34 per cent on day 31, and an insignificant amount on days 36 and 41. Histologic comparisons of tissues showed that the ventral prostates of ordinary castrates were in a more highly stimulated condition than those from castrates that had undergone adrenalectomy, especially when autopsies were carried out on days 26 and 31; on days 36 and 41 differences in tissues from the two types of animals were less conspicuous or were entirely absent. These authors concluded that " . . . at a certain age in the immature rat male the adrenal, in the absence of testes, assumes an andromimetic function." Gersh and Grollman (39) compared the prostates of rats castrated on day 14 with those from adrenalectomized castrated animals, the autopsies being carried out on days 21 and 28. On the basis of volume determination for the glands, and on the basis of the histologic character of the tissues (acinar diameters, cell height) these investigators failed to find any effect from adrenal glands on the development of the accessory reproductive

glands, believing that the secretory development and growth exhibited by the prostate in young castrated rats represented an innate potentiality for growth; they thus failed to confirm the reports of Burrill and Greene.

My own experience with adrenalectomy in prepuberal males is limited to observations on 4 litters of rats castrated and adrenalectomized on days 20, 21 and 25 with autopsies on days 29, 31, 34 and 37. This involved a total of 12 adrenalectomized castrates to be compared with 10 castrates with adrenals intact; comparisons were within each litter, rather than with the group as a whole. Fresh weights of ventral prostates and of seminal vesicles, and histologic study of tissues from all animals were utilized for an evaluation of results. Tissues were dissected as carefully as possible under a binocular microscope and weighed immediately on a torsion balance. Average ventral prostate weights from the 4 different litters ranged between 3.8 and 8.3 mg. and seminal vesicle weights, between 3.2 and 7.7 mg. The actual differences between weights of ventral prostates from ordinary castrates and from adrenalectomized castrates gave an average range of 1.2 to 3.3 mg., and similar differences in seminal vesicle weights were in the range of 0.1 to 2.4 mg. For each animal, actual weights were converted to milligrams of organ weight per 100 grams of body weight. One should consider the handling of such small tissues in attempting to evaluate the significance of fresh weight of organs as evidence in support of a thesis.

For what the data may be worth, Table 4 shows that in adrenalectomized castrated animals, subjected to autopsy on day 29, ventral prostates were 3 per cent lighter in weight and seminal vesicles 50 per cent heavier than the average from litter-mate castrated controls; with autopsy on day 31, ventral prostates were 26 per cent and 38 per cent (DCA-treated) lighter, and seminal vesicles 14 per cent heavier and 4 per cent lighter, respectively, than in the castrated controls; with autopsy on day 34 ventral prostates were 31 per cent lighter and seminal vesicles 12 per cent heavier; and with autopsy on day 37, ventral prostate weights were unmodified but the seminal vesicles were 52 per cent heavier than those from castrated controls.

Histologic study of ventral prostates and seminal vesicles from adrenalectomized castrates and castrated controls served to eliminate seminal vesicles from consideration, since those from all animals showed a basic castrate condition which was without perceptible differences in any group. Ventral prostate glands, on the other hand, from 29-day-old males castrated on day 20 showed a well developed secretory state which would have undergone a decided involution within the course of three to six days. The prostates from adrenalectomized castrates had many acini in good secretory condition (Figs. 10 and 11) and were far from the typical castrate involutionary state that occurs at from 30 to 35 days of age. However, a

careful survey of several preparations convinced us that detectable differences were to be noted and that prostates from adrenalectomized castrates were somewhat inferior to those from castrated controls. Whether this effect resulted from the absence of adrenals or from other changes associated with the body weight loss of 38 per cent in adrenalectomized castrates, is uncertain. This litter was sacrificed at this age range in order to obtain the most clear-cut differences, on account of the impending natural involution of prostates in ordinary castrates. In the litter coming to autopsy on day 34, similar but less apparent differences appeared, again with prostates from castrated controls showing the better condition; but in autopsies performed on days 31 and 37, differences in the state of the ventral prostate were not apparent.

This phase of the present study is regarded as the most unsatisfactory, and the one most open to question or bias, and additional investigations are required before the writer, at least, is convinced that the intact adrenal gland contributes androgens to the otherwise normal animal, or is capable of secreting androgens in the absence of the normal gonad. No evidence was obtained that adrenalectomy in older animals interfered in any way with the attainment of sexual maturity but it should be emphasized that for the most part the experiments reported here were on a relatively short-term basis.

Reichstein (59) obtained adrenosterone, from extraction of adrenal materials. It is a weak androgenic substance when tested on the capon comb. Grollman (60) comments that "This substance occurs in only small amounts in the adrenal and is readily derivable by a hydrolytic process from crystalline substances found in adrenal extracts. It is thus most likely that the occurrence of adrenosterone in adrenal extracts has no physiological significance. It is too feeble an androgen to be the substance responsible for the androgenic action of adrenal-cortical tumors."

Despite the paucity of dependable evidence for the secretion of sex hormone-like substances by the normal adrenal the fact still remains that in man, and in certain strains of mice, adrenal cortical pathology is many times accompanied by modifications attributable to secretions of a sex hormone-like nature. The many cases in human individuals that display changes attributable to sex hormone actions and which are relieved or reversed by removal of cortical tumors, and the nodular umorts that develop in gonadectomized mice associated with an active state of the reproductive tracts, are strong evidence for the participation of the adrenal tissues in the production of the effective substances. Since we do not expect pathologic tissues to maintain a normal metabolism one could speculate at length on the possibility that the pathologic condition may engender an abnormal synthesis of steroids which are usually built into

normal cortical secretions and, instead, produce compounds having androgenic or estrogenic activity. The close chemical similarity of cortical steroids to sex steroids may form the basis for the manufacture of sex-hormone-like substances under the deranged metabolism, suggesting the possibility that similar substances are not formed when normal function prevails.

It would appear that many modifications, reactions or developmental phenomena within the organism are perhaps too freely interpreted on the assumption of adrenal cortical steroid influences, without a clearly established foundation to make plausible the explanations offered. It is the opinion of the writer that more definite information is greatly needed before too much credence can be given to the direct participation of adrenal secretions in reproductive activities. That adrenal secretions are important in maintaining sufficiently normal physiologic conditions within the organism for reproductive function is a well established concept, but that excessive normal cortical secretions have marked influences on sex and reproduction, or that pathologic physiology enhances our conception of the real functional aspects of the adrenal, rests upon assumptions rather than upon well established facts.

VII. SUMMARY AND CONCLUSIONS

1. Testes from 15-day-old rat embryo donors transplanted into the walls of the seminal vesicles of castrated male hosts, secrete androgens that diffuse from the graft and act locally upon seminal vesicle acini; a high secretory state of the acini develops within six to ten days after transplantation and provides a rapid, delicate, and definite test for androgen. Under these conditions androgen is not carried through the general circulation in sufficient quantities to be detected.

2. Adrenal gland transplanted from embryos or from young postnatal donors, persisting as cortex alone or as cortex and medulla, fails to affect the seminal vesicles in castrated rats. The evidence suggests that the living adrenal graft does not secrete androgens.

3. Liver or embryonic ovaries, when transplanted, persist poorly as grafts and do not release androgens.

4. Cortisone acetate in doses up to 5 mg. per day does not exhibit an androgenic, estrogenic or gonadrotropic effect in young or adult rats.

5. ACTH in doses up to 5 mg. per day fails to exhibit a gonadotropic, androgenic or estrogenic effect, regardless of age and sex and whether or not the rats are normal or castrated. No evidence was obtained to suggest that ACTH stimulates the adrenal of normal or of castrated males to secrete sex hormone-like substances.

6. Adrenalectomy in sexually mature males does not modify reproductive

structures. Adrenalectomy and castration of prepuberal males results in slight, and questionable, evidence for an andromimetric effect upon the prostate gland.

7. The question is raised whether adrenal glands under normal conditions secrete sex-hormone-like substances, and whether the pathologic physiology of the adrenal (tumors) may involve atypical synthesis of steroids that account for clinical symptoms.

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Teaching Clinic

THE DIAGNOSIS OF SEXUAL ABNORMALITIES

Three cases are presented to illustrate the differential diagnosis of sexual abnormalities.

Case 1 (T. T.)

This patient was one of three children; a sister of 10 years was entirely normal, a brother of 7½ years had macrogenitosomia precox. There was no history of other sexual abnormalities in the family.

The patient was found to have an enlarged clitoris at birth. She was reared as a girl, and at the age of 2½ years the clitoris was amputated at another hospital. Her early growth and development were entirely normal. At 3½ years pubic hair appeared and increased progressively. At the age of about 4½ years she began to grow taller very rapidly.

When examined at 6 years 4 months her height of 50.5 inches corresponded to that of an 8¾-year-old girl. Her weight was normal for her height. The dental development was normal. Her features were pleasant and feminine. There was sparse coarse hair 2 to 3 cm. in length over the mons veneris and labia majora. A clitoral stump about 1.5×1.0 cm. was palpable. The labia majora were normally formed. On separating them, labia minora were visible. There was a slit-like vulval depression covered with mucous membrane. In the center of this, at the base of the clitoral stump, there was a single orifice about 0.5 cm. in diameter. The nipples and areolae were infantile, and no mammary tissue was palpable. X-ray examination of the bones showed an epiphysial development of 11 years. During the first five days of the hospital study, while the patient had a slight respiratory infection, the urinary 17-ketosteroids ranged from 10.7 to 19.8 mg./day with an average of 14.5 mg./day. During the next four days, after the cold had subsided, the average was 8.8 mg./day.

A urethroscope was introduced into the vulval orifice. At a distance of about 1 cm. a bifurcation was found. Anteriorly the urethroscope was introduced into the bladder. When withdrawn and passed posteriorly, the vagina was entered. At the end of the vagina a cervix was readily visualized.

Comment. The anatomic findings did not exclude the possibility that this patient might be a case of genetic intersexuality with either testes, ovotestes or ovaries; but on the basis of the history of a brother with adrenal hyperplasia and the finding of progressive virilization and high excretion of 17-ketosteroids, a diagnosis of female pseudohermaphrodism due to congenital adrenal hyperplasia was made.

Treatment was begun on January 25, 1952, with 50 mg. of cortisone daily given intramuscularly. By the fourth day the urinary 17-ketosteroid excretion had decreased to 1.6 mg./day. The dose of cortisone was decreased to 50 mg. every second day and later to 75 mg. intramuscularly every fifth day (equivalent to 15 mg. daily). This dose has maintained the 17-ketosteroid output at levels between 1 and 3 mg. per day. There has been no progress of virilization. A small amount of breast tissue has developed. Her height has increased only 1¾ inches in the past year.

Case 2 (E. N.)

This patient was the product of the mother's second pregnancy, the first and third having resulted in miscarriages in the third month, and the fourth being a normal boy

now 3 years old. No members of the family were known to have any sexual abnormalities.

She had been normal at birth with normal female genitalia. Her growth and development were normal until May 1951, when she was 5¾ years old. At that time some fine pubic hair appeared. This increased rapidly and became coarser. In October definite enlargement of the clitoris was noticed and a little acne appeared. She grew rapidly, increasing 7 inches in height between May and January.

In January 1952, at the age of 6 years 4 months, she was admitted to the Mayo Clinic. Her height of 49 inches corresponded to that of an average 8-year-old girl. Her weight (55.5 pounds) was 3.5 pounds in excess of the average for her height. The musculature was well developed and there was no excess fat. The dental development was normal for her age. There was abundant coarse hair, 3 to 5 cm. in length, over the labia majora and the pubis, and some sparse hair in the axillae. On separating the labia majora, a slightly enlarged clitoris measuring 2.5 cm. in length and 1.0 cm. in diameter was seen. The glans was visible. The vulva was of normal female configuration with normally formed infantile labia minora and separate urethral and vaginal orifices. The nipples and areolae were undeveloped and no mammary tissue was palpable. X-ray examination of the bones showed an epiphyseal development of 11 years. Intravenous pyelograms revealed no abnormality.

A number of determinations of urinary 17-ketosteroids showed an output of 12 to 15 mg. per day. Results of various laboratory tests were negative. The patient was given 100 mg. of cortisone intramuscularly daily for four days, during which time the ketosteroid output decreased as follows: 14.5–9.1–4.8–3.8 mg. per day.

Comment. The history of the onset and rapid progress of virilization beginning at the age of 5¾ years in a previously normal girl strongly suggested the possibility of adrenal tumor, but the marked decrease in the urinary 17-ketosteroids with cortisone indicated that the patient had adrenal hyperplasia rather than tumor. This was confirmed by exploration.

Treatment. An exploratory laporatomy was performed and both adrenal glands were visualized. No tumor was found. Both glands were found to be moderately enlarged, and a biopsy revealed adrenal hyperplasia. A normally formed uterus, tubes, and ovaries were present.

Following operation the patient was treated with oral cortisone, 37.5 mg. daily (12.5 mg. three times a day). She moved to Washington and has been followed in our clinic, where treatment was changed to Hydrocortisone (compound F) in a dose of 12.5 mg. given orally three times a day. The 17-ketosteroid output has remained suppressed to levels between 0.6 and 4.0 mg. per day, with an average of 2.1 mg./day. There has been no progress of virilization. A small amount of breast tissue has developed. In the past ten months she has grown 2 inches in contrast to 7 inches in the eight months prior to treatment.

Case 3 (P. C.)

This patient was first seen at the age of 3 years. She had a normal brother of 5½ years. There was no history of sexual abnormalities in the family.

At birth an enlarged hypospadic phallus was noted but otherwise the external genitalia seemed to be of female type. It was decided to raise her as a girl. Her growth and development had been normal in every way. No pubic hair had appeared.

When examined at the age of 3 years, she seemed to be an attractive little girl with long blond hair. Her height of 38 inches corresponded to the average for a 3¼-year-old girl. Her musculature was not overdeveloped. X-ray examination revealed an epiphyseal development very slightly advanced for 3 years. The phallus, measuring 3.5×1.4 cm., resembled a hypospadic penis with well developed glans. It was bound down in a position of chordee by two cord-like structures extending backward toward the sides of a

vulva-like depression. On either side there were fleshy labial or scrotal folds which were unfused even at the posterior end. No solid body was palpated either in these folds or in the inguinal canals. In the vulva-like depression there were two distinct orifices of almost equal size, each measuring about 3 mm. in diameter and separated by a narrow septum. Urethroscopic examination by Dr. Erasmus Kloman showed that the anterior one was the urethra. The posterior orifice was the vagina, at the end of which a definite cervix could be seen. Two determinations of the urinary 17-ketosteroids revealed values of 0.96 and 0.88 mg. per day.

Comment. Although the anatomic structure of the external genitalia was entirely compatible with female pseudohermaphrodism due to congenital adrenal hyperplasia, it was felt that this diagnosis could be excluded because the patient had no evidence of excessive androgen secretion, such as rapid somatic growth, accelerated osseous development, precocious sexual hair or increased 17-ketosteroids. It was believed that this was a case of intersexuality due to a genetic abnormality. Although the patient might have intra-abdominal gonads consisting either of testes, ovotestes or ovaries, in view of the relative infrequency of the occurrence, the presence of true ovaries seemed least likely.

Treatment. An exploratory laparotomy was performed by Dr. Kloman. A normally formed infantile uterus and fallopian tubes were found. In the broad ligament on each side in the usual position of ovaries, there was an oval, white, glistening body. Most of the observers thought these were ovaries. Biopsies with frozen sections were made while the patient was on the table. These showed the typical structure of immature testes; no ovarian tissue was seen. Later a small amount of ovarian tissue was found in the cortex of the left gonad, but none was found in the right.

Since this child had been raised as a girl and since she had a normally formed female genital tract and the phallus was too small to function as an adequate penis, it was decided that her sex should not be altered to correspond to the gonads. Accordingly, the testes were removed, in order to prevent the possibility of masculinization at the age of puberty, and the clitoris was extirpated. During the ensuring two years her growth and development were entirely normal and no secondary sex characteristics appeared. It is planned to give her estrogenic therapy at puberty.

DISCUSSION

The essential findings in the 3 cases presented may be summarized as follows:

	Case 1 (T. T.)	*Case 2 (E. N.)*	*Case 3 (P. C.)*
Family history	Brother with adrenal hyperplasia	Negative	Negative
Genitalia at birth	Enlarged phallus	Normal female	Enlarged phallus
Time of appearance of pubic hair	3½ yrs.	5¾ yrs.	—
At time of examination			
Chronologic age	6 yrs. 4 mos.	6 yrs., 4 mos.	3 yrs.
Height age	8¾ yrs.	8 yrs.	3¼ yrs.
Bone age	11 yrs.	11 yrs.	3¼ yrs.
Clitoris	Amputated	2.5×1.0 cm.	3.5×1.4 cm.
Vaginal and urethral orifices	Typical urogenital sinus	Normal vagina and urethra	Junction at orifice
17-KS excretion	7.0–19.8 mg./day (av., 9 mg.)	12–15 mg./day	0.9 mg./day
Suppression of 17-KS with cortisone	+	+	Not indicated

The differentiation of congenital adrenal hyperplasia in females from other types of sexual abnormalities cannot be made on the basis of the anatomic findings. The external genitalia of female pseudohermaphrodites with congenital adrenal hyperplasia may vary considerably. They all show some enlargement of the clitoris at birth. Although most patients show completely separated labia majora and a vulvar depression or groove into which the urogenital sinus opens as a single urethral-like or funnel-shaped orifice, occasionally there are separate urethral and vaginal openings. In some patients, however, the labia majora are more or less completely fused in the midline and the urogenital sinus may resemble a hypospadic urethra situated on the median raphe. Any type of external genitalia, or of genital duct formation, may be seen in cases of genetic intersexuality. Therefore, the diagnosis of the adrenogenital syndrome must depend upon evidences of increased androgen secretion, namely, increased urinary 17-ketosteroids, excessive somatic growth, accelerated epiphyseal development and precocious pubic hair. The increased ketosteroids can be demonstrated in the earliest months of life, although the other manifestations of androgen may not be evident for three or four years. The ability of cortisone to suppress the excretion of 17-ketosteroids in cases of adrenal hyperplasia serves to differentiate these cases from those of adrenal tumor, in which cortisone has no suppressive effect. The differential diagnosis of the adrenogenital syndrome in females is shown in Table 1.

TABLE 1. DIAGNOSIS OF ADRENAL HYPERPLASIA AND TUMOR IN FEMALES

	Abnormality of embryonic sex differentiation	Evidence of excessive androgen			DHIA	Response to cortisone
		Excess growth and osseous develop.	Precocious sexual hair	17-KS		
Congenital adrenal hyperplasia in females	Hypertrophied clitoris Urogenital sinus	+	+	+	0?	+
Genetic intersexuality with testes, ovotestes, or ovaries	Various abnormalities	0	0	Normal	0	
Adrenal tumor in females	None Later virilization and hirsutism	+	+	+	+?	0

In *Case 1* the diagnosis of female pseudohermaphrodism due to congenital adrenal hyperplasia was in no doubt. A history of siblings having the same condition or having male macrogenitosomia precox is often a great aid to diagnosis. The patient presented the usual anatomic findings at birth. The diagnosis probably could have been confirmed within the first month or two of life by demonstrating increased urinary 17-ketosteroid excretion. At that age the output would probably have been 2–4 mg. per day. We would have accepted any increase over 2 mg. per day as diagnostic, particularly if the administration of cortisone caused a suppression to a level of 0.5–1.0 mg. per day. We would not consider exploration of the adrenals or exploratory laporatomy necessary or desirable for diagnosis in this case. The patient followed the usual course and the appearance of sexual hair at the age of $3\frac{1}{2}$ years, together with accelerated growth and osseous development, would have made the diagnosis of the adrenogenital syndrome certain, even without knowledge of the level of 17-ketosteroid excretion.

Case 2 presented an interesting diagnostic problem. This patient showed no anatomic abnormality at birth. The first evidences of virilization appeared at the age of $5\frac{3}{4}$ years and progressed rapidly. This would have suggested strongly that she had an adrenal tumor. I am not aware of any other cases in which adrenal hyperplasia began after birth and before the age of puberty, nor does Dr. Randall Sprague of the Mayo Clinic know of such cases. Of course, after the age of puberty and in adults, virilization is frequently due to adrenal hyperplasia rather than to tumor. The growth of pubic hair in this child at the age of $5\frac{3}{4}$ years might have been due to "premature pubarche" (or "precocious adrenarche" as Talbot calls it), except that the hypertrophy of the clitoris, rapid acceleration of growth and osseous development, and the marked increase in urinary 17-ketosteroids excludes this diagnosis. In "premature pubarche," which is an entirely benign condition, there is an early growth of sexual hair, with only slight increase in the 17-ketosteroids (to levels of 2–5 mg. per day), but no progressive or rapid virilization. Accordingly, I would have suspected strongly that this patient had a virilizing adrenal tumor, but I would have tested the response of the 17-ketosteroids to the administration of cortisone, as was done at the Mayo Clinic. The rapid fall of the urinary 17-ketosteroids within a few days was strong evidence against adrenal tumor. I believe now that in the adrenogenital syndrome (but not in Cushing's syndrome) the differentiation between adrenal tumor and hyperplasia can be made on the basis of the cortisone test, without surgical exploration. However, the onset of adrenal hyperplasia is so rare at the age of this patient that I would have wanted to explore her adrenals to confirm the validity of the test.

Case 3 illustrates the way in which the differentiation between genetic intersexuality and female pseudohermaphrodism due to congenital adrenal hyperplasia can be established without surgical exploration. This patient presented practically the same abnormalities of her sex organs at birth as did Patient No. 1. In fact, surgical exploration later revealed that her entire genital tract was the same, except for the gonads. However, at the age of 3 years she had shown no evidence of virilization. With congenital adrenal hyperplasia, pubic hair occasionally appears somewhat later than this. On the other hand, if there had been hyperadrenocorticism her height and bone age would have been increased by that time. The finding of low urinary 17-ketosteroids definitely excluded the possibility. In such cases of genetic intersexuality, surgical exploration and gonadal biopsies are essential to determine the nature of the gonads and the structure of the genital tract. I believe that the decision as to the sex in which the patient is to be reared and as to the surgical corrections to be made, depends to a considerable extent upon the structure of the external genitalia. It is foolish to raise as a male an individual with testes, who may have only a tiny phallus but a well developed vagina or vaginal pouch. The decision should be made in early infancy and changes in sex in later life should be avoided if possible.

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Editorial

JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM

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PRESENT STATUS OF THE ENDOCRINE TREATMENT OF ADVANCED PROSTATIC CANCER

IT HAS now been over ten years since Huggins postulated a new concept for the management of patients with prostatic cancer. The results of his investigations revealed that some prostatic cancers are influenced by the presence of androgens in the body and that the neutralization or inactivation of these androgens will produce a retrogression in the growth of the tumor. This can be brought about by removing the principal source of the production of androgens, *viz.*, the testis, by orchiectomy, or by the administration of estrogenic substances, or both. This new concept for the treatment of this disease was accepted with a great deal of enthusiasm because of the discouraging results that had previously been obtained from all forms of therapy. Enough time has now elapsed so that the physician has been able to appraise the results of this form of therapy and the original enthusiasm has been tempered by the knowledge gained in the last ten years. It is now known that although the endocrine treatment of prostatic cancer is far superior to all previous forms of therapy, the beneficial results are not universal, the duration and degree of improvement is variable, and eventually a majority of patients experience a relapse and succumb from the disease.

In order to evaluate better the therapeutic results of orchiectomy, estrogen therapy, or a combination of the two in the treatment of advanced prostatic cancer, Nesbit and Baum collected data on 1,818 cases from a group of urologists on the North American continent. For the purpose of standardization, a prescribed printed form was employed for the collection of information. Although the survival statistics were based on an examination of 1,818 patients, the actual number of patients included in the series was 1,554, of which 947 were followed for three years and 607 for five years. The survival rates in these two groups were compared with the rates in a

group of 795 untreated patients followed for a similar period of time. It was their conclusion from this study that patients with prostatic cancer who respond favorably to castration or estrogen therapy, or both, live more comfortably and longer than patients not so treated. Patients who fail to show a response have survival rates that are identical to those of the untreated group. The five-year control of prostatic cancer is more effectively obtained by combined treatment with orchiectomy and stilbestrol in patients who are free of metastases. When metastases are present, orchiectomy is more effective than estrogens; the combination of stilbestrol and orchiectomy does not appear to offer any advantage over orchiectomy in this group of patients. The maximum benefit occurs when treatment is started as soon as the diagnosis is made. In the management of the patient who has a relapse there is no advantage of one form of therapy over the other.

In a recent report by Huggins, it is observed that when anti-androgenic treatment is used in cancer of the prostate the results fall into three categories: 1) no effect upon the cancer, 2) regression of the primary neoplasm and its metastases, and 3) retrogression of the primary neoplasm with progression of metastases. Moreover, the prostatic cancer has lost so many of the qualities of the normal prostatic epithelium that its metabolism is self-regulating, and therefore is is not influenced favorably by hormonal therapy.

Huggins further has demonstrated that certain mammary and prostatic cancers are dependent on the function of the adrenal and that some patients with widespread cancer have an amelioration of the disease after total bilateral adrenalectomy. However, many patients were not benefited in any way by this form of therapy and there was a stimulation of the cellular growth, particularly of melanosarcoma, chorionepithelioma of the testis, cancer of the lung and stomach, and squamous-cell carcinoma. He has also observed that some patients with advanced prostatic cancer, who suffer a relapse after anti-androgenic therapy, may be benefited by the administration of 5 mg. of stilbestrol and 50 mg. of cortisone a day.

It must be emphasized that it will do little good to attempt any form of endocrine therapy in a patient with bladder insufficiency and residual urine caused by the prostatic cancer. The obstruction must be relieved and renal function restored. This can be accomplished best by transurethral resection.

There are still many questions that remain unanswered in the endocrine treatment of prostatic cancer. Great progress has been made in the treatment of this disease, however, and as a result many patients have been given a longer and more comfortable span of life.

NORRIS J. HECKEL, M.D.

Questions and Answers

(Answers by Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore, Md.)

QUESTION: Do you recommend any special treatments in premature and excessive development of breasts in girls?

ANSWER: No. Breast development without other estrogenic manifestations is not uncommon in young girls long before the onset of puberty. It may disappear spontaneously after a year or more. There is no satisfactory treatment for the "constitutional" type of true sexual precocity with breast development.

QUESTION: Are EKG changes of any value as a diagnostic aid in myxedema?

ANSWER: In hypothyroidism the EKG frequently reveals decreased voltage of both the QRS and T-wave deflections. Occasionally there is a prolonged QRS interval with evidence of interventricular block. However, the diagnosis should not be based on the presence or absence of these changes. In hypothyroidism there is widespread impairment of various physiologic and chemical functions and, during childhood, marked retardation of growth and development.

QUESTION: Are hypothyroidism and myxedema the same?

ANSWER: Many writers use these terms interchangeably. However, the term "myxedema" should be limited to those cases of hypothyroidism in which there are clinical evidences of subcutaneous myxedematous deposits. Hypothyroidism can exist without apparent myxedema.

QUESTION: What dose of desiccated thyroid do you use in myxedema; is there any relation to the weight and age?

ANSWER: During childhood, normal growth and development depend upon giving sufficient replacement therapy to bring about euthyroidism. In infants under 2 years of age this requires doses of $\frac{3}{4}$ to 1 grain daily (45 to 60 mg.) of U.S.P. desiccated thyroid. Older children require from $1\frac{1}{2}$ to $2\frac{1}{2}$ grains (90 to 150 mg.). It is important to start treatment gradually, beginning with $\frac{1}{4}$ grain (15 mg.) in infants and $\frac{1}{2}$ grain (30 mg.) in older children and increasing by these increments at intervals of three weeks.

In adults who may have cardiovascular disorders, even greater caution is advisable and sometimes they are better left in a slightly hypothyroid state. Most adults are well maintained on 1 to 2 grains (60 to 120 mg.) daily.

QUESTION: Please review the differential diagnosis of mongolism *vs.* cretinism at birth.

ANSWER: The mongoloid can usually be recognized at birth because of characteristic anatomic abnormalities. The eyeslits are slanted slightly upward. More characteristic than this is the shortness of the palpebral fissure, causing the eyes to appear small. In most cases there is an epicanthal fold. The head is smaller than average and is usually, although not invariably, brachycephalic with a markedly undeveloped occiput. The ears are generally small, often misshapen, and placed low on the skull in relation to the

orbit. The hands and fingers are usually short and broad and there may be a short, incurving fifth finger. There is hyperextensibility of the joints. The mongoloid may be reasonably active and the skin may be warm, moist and of good color. There is no special tendency to constipation.

The cretin may not present a characteristic appearance at birth, but he fails to grow and develop normally. At birth he may be somewhat puffy, with loose flabby tissues and the tongue may be large, broad and thick. These features become more pronounced as he grows older. He is slow, sluggish, sleeps considerably and cries little. He is difficult to feed and is usually constipated. The abdomen may be distended and there may be an umbilical hernia. The skin is usually of a pale, dusky gray color and may show marked dusky mottling and feel cool on exposure. The dusky cyanosis may even arouse a suspicion of cardiac disease.

QUESTION: I have never seen a blond cretin or a dark-haired mongoloid. Are there other observations of this phenomenon?

ANSWER: No. Both cretinism and mongolism may occur in either blonds or brunettes.

(*Answers by Dr. Samuel F. Haines, Mayo Clinic, Rochester, Minn.*)

QUESTION: What is your opinion on the use of desiccated thyroid and Lugol's solution in the treatment of malignant exophthalmos?

ANSWER: Occasionally severe exophthalmos has regressed during the administration of Lugol's solution and desiccated thyroid. On the other hand, in some instances, the condition has progressed rapidly to serious proportions. The fluctuating nature of the condition, its relative infrequency, and the urgency for treatment when it does occur, with the resultant institution of many therapeutic measures simultaneously, make the evaluation of any form of treatment difficult. The abolition of some of the ocular signs of Graves' disease by the administration of Lugol's solution and the tendency for proptosis of the globes to be slightly greater after control of the hyperthyroidism of Graves' disease than before (Soley, Dobyns) indicate the probable desirability of the simultaneous administration of Lugol's solution and desiccated thyroid in patients with severe exophthalmos, although no unequivocal proof of the value of this procedure has yet been produced.

QUESTION: With current concepts of TSH in the production of exophthalmos, why don't all myxedema patients have exophthalmos?

ANSWER: The fact that the urinary excretion of TSH is usually greater than normal in patients with myxedema and that in most of these patients exophthalmos does not occur, constitutes one of the most valid objections to assuming that TSH is in itself responsible for the production of exophthalmos. The most potent arguments linking increased TSH production with exophthalmos are, 1) the finding of increased TSH excretion in the urine in some patients with exophthalmos (a finding opposite to the usual occurrence in Graves' disease), and 2) the experimental induction of exophthalmos following administration of TSH, particularly in the thyroidectomized animal. The contrary implications of the foregoing observations make it clear that our present knowledge of the etiology of exophthalmos is very far from complete.

QUESTION: A 14-year old male underwent neck dissections four years and three years

ago for papillary thyroid carcinoma, and was subsequently given 1 grain of thyroid daily. Diabetes mellitus developed two years ago, with an insulin requirement of 70 units daily. At this time pulmonary metastases were evident and have remained unchanged in appearance. He is in apparent good health. Should the metastases be treated?

ANSWER: The fact that the patient appears in good general health despite the presence of pulmonary metastases is not an unusual observation in such cases. However, if the diagnosis is correct, it can be assumed that with progression of the disease, increasing difficulty and ultimate death will occur; therefore, it seems imperative that every effort be made to treat the pulmonary metastases. The most effective treatment, if it is suitable in other regards, would be by the administration of radioactive iodine (I^{131}). Administration of desiccated thyroid should be discontinued and after a suitable period of time a radioiodine tracer should be given to determine whether or not there is any collection of radioiodine by the metastatic lesions and also whether any functioning thyroid tissue remains in the neck. After complete eradication of functioning thyroid tissue in the neck, if no collection, or a very small collection, of radioiodine occurs in the metastatic lesions, an attempt should be made to stimulate the collection by the prolonged administration of thiouracil, or the administration of TSH, or both. Providing an adequate collection of radioiodine occurs, large doses of I^{131} should be given. If no collection of radioiodine occurs, or can be induced, reliance must then be placed upon roentgen therapy which, in lesions of this type, is ordinarily inadequate and unsatisfactory for therapeutic purposes. The development of diabetes mellitus in this patient is probably of no significance in relation to the thyroid disease.

QUESTION: What is the best way of achieving reduction of exophthalmos in patients with Graves' disease 1) with increased BMR; 2) with decreased BMR?

ANSWER: There are no satisfactory methods at the present time for achieving reduction of exophthalmos in patients with Graves' disease. In those patients with elevated basal metabolic rates, hyperthyroidism should be controlled by whatever means may be necessary; care should be taken, however, that the basal metabolic rate is not allowed to fall below the average normal level. In those patients with decreased basal metabolic rates, both Lugol's solution and desiccated thyroid should ordinarily be given and the level of the basal metabolic rate maintained at or about normal. The patient should sleep with the head elevated to as great a degree as is possible to avoid the added accumulation of orbital edema as the result of gravitational changes. In either case the treatment of exophthalmos will depend upon its severity. Favorable results have followed roentgen treatment to the orbits, roentgen treatment to the pituitary, tarsorrhaphy and, in severe cases, orbital decompression as described first by Naffziger; but after none of these procedures have universally good results been obtained. The choice of treatment must in all cases be dependent upon the seriousness of the condition, but it is frequently very difficult to determine the exact point in the course of the condition at which more radical procedures should be instituted.

(Answers by Dr. E. C. Reifenstein, Jr., Oklahoma Medical Research Institute and Hospital, Oklahoma City, Okla.)

QUESTION: How stable is the Sulkowitch reagent?

ANSWER: If properly prepared and placed in a clean container, the Sulkowitch reagent should be stable indefinitely. If sufficient calcium is added to the solution, however, there will be some precipitation.

QUESTION: How do you treat senile osteoporosis in males?

ANSWER: Senile osteoporosis in males is treated with exactly the same regimen as is used in treating either senile or postmenopausal osteoporosis in females. The most important medication for therapy of this condition is some form of estrogen. For example, one can give orally "Premarin," 1.25 mg. three times a day for four weeks, skip seven days, and repeat; or one can give injectible estrogens in equivalent dosage. It is important to emphasize that estrogenic medication should always be given intermittently, even in males. That is, the estrogens should be given for four weeks, then stopped for a week, in order to eliminate over-stimulation of the male breast. The estrogenic therapy must be continued for a considerable period of time, at least for five to ten years.

Second on the therapeutic program is the administration of some form of androgen. For example, androgen can be administered as testosterone "linguosorbs" (6 mg. three times a day) for absorption through the buccal membranes of the mouth; as methyltestosterone (10 mg. daily orally), as testosterone suspension (10 mg. twice a week, by injection), or as testosterone pellets (75 mg. every three months, by implantation). The androgenic therapy should be given continuously for at least a year and preferably for as long as the estrogen is continued.

The estrogenic and androgenic steroid medications have an effect upon extracellular fluid as well as upon anabolism of protoplasm and bone. If edema develops, the first step is to reduce the amount of sodium in the intake. If this does not control the edema, then the patient should be given ammonium chloride or mercurial diuretics. Finally, if the edema persists after these measures have been tried, it may be necessary to reduce the steroid dosage.

There are other important points in the therapeutic program for treating patients with senile osteoporosis. They should eat a high-protein diet to provide materials with which they can build the protein matrix or osteoid of bone. They should take an adequate amount of water in order to avoid any tendency toward a concentrated urine, when hypercalciuria is present. An excess of calcium or vitamin D is to be avoided and can be very harmful. These patients do not need extra calcium or vitamin D, as their primary need is for osteoblastic activity and for protein constituents. Furthermore, an excess of calcium or of vitamin D may aggravate the hypercalciuria and lead to renal calculi. Excessive immobilization must be avoided, so that atrophy of disuse is not added to the causative factors for the osteoporosis. At the same time, it is desirable to provide some support for collapsed vertebrae, and the use of a corset or brace supporting these mechanically defective areas is worthwhile. It is helpful, also, to teach the patient the correct use of his muscles, in order to avoid an acute strain of the weakened back. It is necessary to bear in mind that these patients may have renal calculi, and that these or the accompanying urinary tract infections may require therapy.

QUESTION: By means of injections of insulin in addition to glandular treatment, can you accelerate or retard growth in children in whom the epiphyses are open?

ANSWER: One must begin by pointing out that insulin is, after all, a hormone and is therefore a form of glandular treatment. However, in my opinion, there is no evidence that it has any direct effect upon the rate of growth in children who have open epiphyses. It does have an indirect effect which may be marked. Small doses of insulin are a potent stimulus to the appetite; with an increased intake of foodstuffs (particularly protein) and calories, growth may be accelerated in children who have open epiphyses and who have previously not been eating a sufficient amount of food.

The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9,* in the Drake Hotel, Chicago, Illinois.

THE LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the AAAS will hold its 1953 annual meeting at Mont Tremblant Lodge, Mont Tremblant, Quebec, during the period September 6–11. Interested investigators and specialists in the hormone field may apply for attendance by writing to the Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Mass., for application blanks. Since accommodations at the hotel necessarily limit the attendance, only those persons submitting applications can be considered. The application blanks must be received by the Committee no later than *May 15* in order to insure issuance of invitations as soon as possible thereafter.

The following program has been arranged:

I. *Nervous system-hormone interrelationships*

The central nervous system and stress-induced eosinopenia.

Dr. R. W. Porter, Univ. of Calif. School of Medicine at Los Angeles

Studies of brain metabolism and electrical activity in relation to adrenocortical physiology.

Dr. Hudson Hoagland, The Worcester Foundation for Experimental Biology

Effects of hormones on cerebral function.

Dr. D. M. Woodbury, Univ. of Utah School of Medicine

II. *Thyroid hormone physiology and biochemistry*

Triiodothyronine in relation to thyroid physiology.

Drs. J. Gross and R. Pitt-Rivers, National Inst. for Medical Research, London, and State Univ. of New York Medical Center

Metabolic effects of thyroid hormones *in vitro*.

Drs. H. A. Lardy and G. Feldott, Univ. of Wisconsin School of Medicine

III. *Comparative endocrinology*

Endocrine mechanisms in the life of insects.

Dr. D. Bodenstein, U. S. Army Chemical Center, Maryland

Hormones produced by neurosecretory cells.

Drs. Ernst and Berta Scharrer, Univ. of Colorado Medical Center

IV. *Protein hormones*

The preparation and chemistry of crystalline insulin.

Dr. R. G. Romans, Connaught Medical Research Laboratory

The chemistry of ACTH.

Drs. E. E. Hays and W. F. White, The Armour Research Laboratories

V. *The role of hormones in blood and blood-forming organs*

Endocrine factors and radiation-induced lymphoid tumors of mice.

Dr. Henry S. Kaplan, Stanford Univ. School of Medicine

Endocrine influences upon the formed elements of blood and blood-forming organs.

Dr. Albert S. Gordon, Washington Square College, New York Univ.

VI. *Aspects of clinical endocrinology*

Some problems related to ovarian function and to pregnancy.

Dr. B. Zondek, Rothschild Hadassah Univ. Hospital, Jerusalem

Clinical studies on electrolyte and fluid metabolism.

Dr. Rolf Luft, Serafimerlasarettet, Stockholm

Endocrine-metabolic studies in man.

Dr. J. W. Conn, Univ. of Michigan School of Medicine